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**A THESIS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY**

Chemical-based management program against *Bemisia tabaci*, *Spodoptera litura*, and *Frankliniella occidentalis* using selective insecticides to seven commercialized biological control species in greenhouses

시설재배에서 선택적 살충제를 이용한 담배가루이,
담배거세미나방 및 꽃노랑총채벌레의 화학적
방제 프로그램

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Seoul National University
August 2019**

Chemical-based management program against *Bemisia tabaci*, *Spodoptera litura*, and *Frankliniella occidentalis* using selective insecticides to seven commercialized biological control species in greenhouses

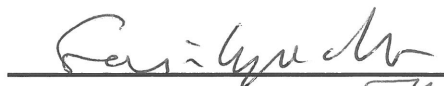
**UNDER THE DIRECTION OF ADVISER JOON-HO LEE
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF SEOUL NATIONAL UNIVERSITY**

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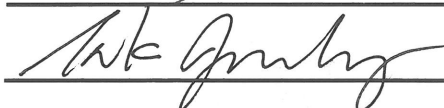
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Abstract

Chemical-based management program against *Bemisia tabaci*, *Spodoptera litura*, and *Frankliniella occidentalis* using selective insecticides to seven commercialized biological control species in greenhouses

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Controlling *Bemisia tabaci* (Gennadius), *Spodoptera litura* (Fabricius), and *Frankliniella occidentalis* (Pergande), which are dominant pests in fruit vegetables in greenhouses, is a key factor to increase the crop yield. For controlling the population and insecticide resistance of these pests, two complementary strategies of selective insecticides and biological control agents have been suggested. The objective of this study was to suggest effective and selective insecticides against biological control species to establish a greenhouse IPM program.

To select effective insecticides, with their information on mode of action, their toxicities were evaluated against egg, nymphal, and adult stages of *B. tabaci*, larval stage of *S. litura*, and adult stage of *F. occidentalis*. Three avermectins (abamectin, emamectin benzoate, and lepimectin), 1 METI acaricide (pridaben), 1 neonicotinoid (dinotefuran), and 1 spinosyn (spinetoram) were selected against all the stages of *B. tabaci*. Four insecticides (chlorantraniliprole, cyantraniliprole, flubendiamide and indoxacarb) were selected *S. litura* showing high residual activities until 21 days. Showing residual activities until 7 days, 6 insecticides (bistrifluron, chlorfenapyr, methoxyfenozide, chlorpyrifos, bifenthrin, and spinetoram) were selected as alternative insecticides for controlling resistance. Three insecticides (spinetoram, spinosad, and emamectin benzoate) were selected showing high contact and ingestion toxicities to *F. occidentalis* adults, and chlorfenapyr was selected as an alternative insecticide with high ingestion toxicity.

To select insecticides compatible with biological control species, the toxicities of 13 insecticides on 2 predatory mites (*Phytoseiulus persimilis* Athias-Henriot and *Amblyseius swirskii* Athias-Henriot), 2 hemipteran predators (*Orius laevigatus* [Fieber] and *Nesidiocoris tenuis* Reuter), and 3 hymenopteran parasitoids (*Diglyphus isaea* [Walker], *Aphidius colemani*

Viereck, and *Encarsia formosa* Gahan) were compared in direct and residual applications in the laboratory. Six insecticides (dinotefuran, indoxacarb, chlorantraniliprole, cyantraniliprole, methoxyfenozide, and bistrifluron) were safe to predatory mites, and 3 insecticides (methoxyfenozide, bistrifluron, and chlorantraniliprole) were safe to both hemipteran predators. One insecticide (chlorantraniliprole) was selected against *D. isaea*.

To find the effective insecticide application methods, I compared the efficacies of three application methods, foliar spray, foliar spray mixed with a wetting agent, and soil drenching, on the red pepper, *Capsicum annuum* plants, and compared the residue of chlorantraniliprole on their leaves and fruits. The highest efficacy was found in the foliar spray followed by the foliar spray mixed with a wetting agent and soil drenching application methods. However, residue of chlorantraniliprole in the foliar spray began to decline quickly than the residue of other application methods. The efficacy and the residue in the foliar spray and the foliar spray mixed with a wetting agent remained stable until 42 days. The residue and efficacy in soil drenching were low until 3 days, but the residue increased and continued until 42 days. The foliar spray should be selected in high population of lepidopterans due to the efficacy in early days after application. The soil drenching should be

selected in low population of the insects due to the residual efficacy after 3 days of application.

In this study, 6 insecticides on *Bemisia tabaci*, 10 insecticides on *S. litura*, and 4 insecticides on *F. occidentalis* were selected. Chlorantraniliprole was safe either after or before the release of 7 biological control species (2 predatory mites, 2 hemipteran predators, and 3 hymenopteran parasitoids). The foliar spray of chlorantraniliprole was the most effective application method as it showed highest efficacy and residue.

Key words: Integrated pest management, selective insecticide, efficacy, resistance, biological control species, application method

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Chapter 1.

General Introduction

Cultivation area of greenhouse fruit vegetables decreased gradually from 2014 to 2018 with an average annual growth rate of -5.2%. However, the annual growth rates of radish, cucumber, potato and green pepper have been increased 9.9, 6.1, 2.5, and 1.0%, respectively (Fig. 1.1). Cultivation area of leaf vegetables also decreased with an annual growth rate of -3.0%. In many fruit vegetable cultivation systems, *Bemisia tabaci* (Gennadius), *Spodoptera litura* (Fabricius), and *Frankliniella occidentalis* (Pergande) cause significant damages in South Korea (Chung, 2001; Park et al., 2002; Park et al., 2010; Lim et al., 2012).

Bemisia tabaci is a serious insect pest making direct damage by sucking plant sap and indirect damage by sooty mold infection or virus-vectoring on fruit vegetables in greenhouse (Kontsedalov et al., 2008; Helmi, 2010; Cameron et al., 2016).

Spodoptera litura has a wide host range of more than 120 species (leguminosae, cruciferae, solanaceae, gramineas, and other ornamentals) and causes a serious direct damage on fruits and leaves of vegetables in greenhouse (Cho et al., 1996; Bae et al., 2007; Ahmad et al., 2008; Kim et al., 2009; Lim et al., 2012; Muthusamy et al., 2014).

In South Korea, invasive thrips have been observed since 1990s: *Thrips simplex* (Morison) in 1991, *F. occidentalis* in 1993, and *Thrips palmi*

Karny in 1993 (Lee et al., 2003). *Frankliniella occidentalis* is a serious insect pest having a wide host range of horticultural and ornamental crops and causes direct sucking damage and disease by virus transmission of tospoviruses such as tomato spotted wilt virus (TSWV) in greenhouse (Chung, 2001; Park et al., 2002; Jeon and Kim, 2006; Park et al., 2009; Jacobson et al., 2013). To increase the yield of crops by reducing the population of *B. tabaci*, *S. litura*, and *F. occidentalis*, farmers are spraying repetitively insecticides as a primary strategy (Lee et al., 2002; Anmad et al., 2008; Rahman et al., 2012). But, the repeated spray and overuse of insecticides have caused the development of insecticide resistances to numerous insecticides and the disruption of beneficial insects. The failure of chemical control by the development of resistances may conduce to more serious chemical abuse (Ahmad, 2007; Basit et al., 2011; Lee et al., 2012).

Therefore, the objective of this study was to develop management program using ecologically selective pesticides against the populations of *B. tabaci*, *S. litura*, and *F. occidentalis* with biological control species.

In this thesis, the management program was presented as a diagram (Fig. 1.2). The selection of effective insecticides against those insect pests was made in Chapter 2. For IPM system, selective toxicities of the 13 insecticides were evaluated against biological control species (2 predatory

mites, 2 hemipteran predators and 3 hymenopteran parasitoids) from direct and residual efficacies in Chapter 3. In Chapter 4, the effective application methods were studied. For the effective adoption in greenhouse cultivation system, application methods including foliar spray of insecticide only, foliar spray mixed with a wetting agent, and soil drenching around the root of crops were tested on the red pepper (*Capsicum annuum*) plants and compared.

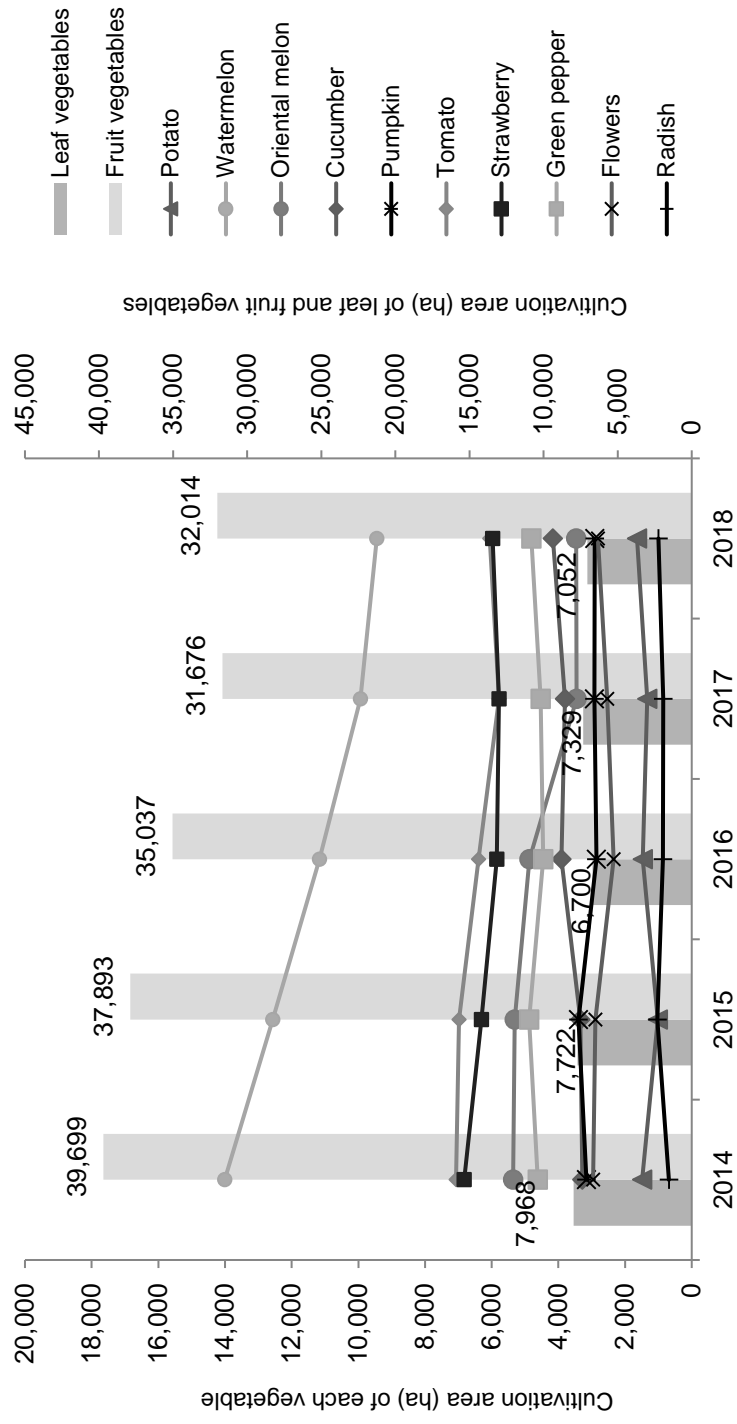


Fig. 1.1. Cultivation area of fruit and leaf vegetables in greenhouse cultivation system in South Korea.

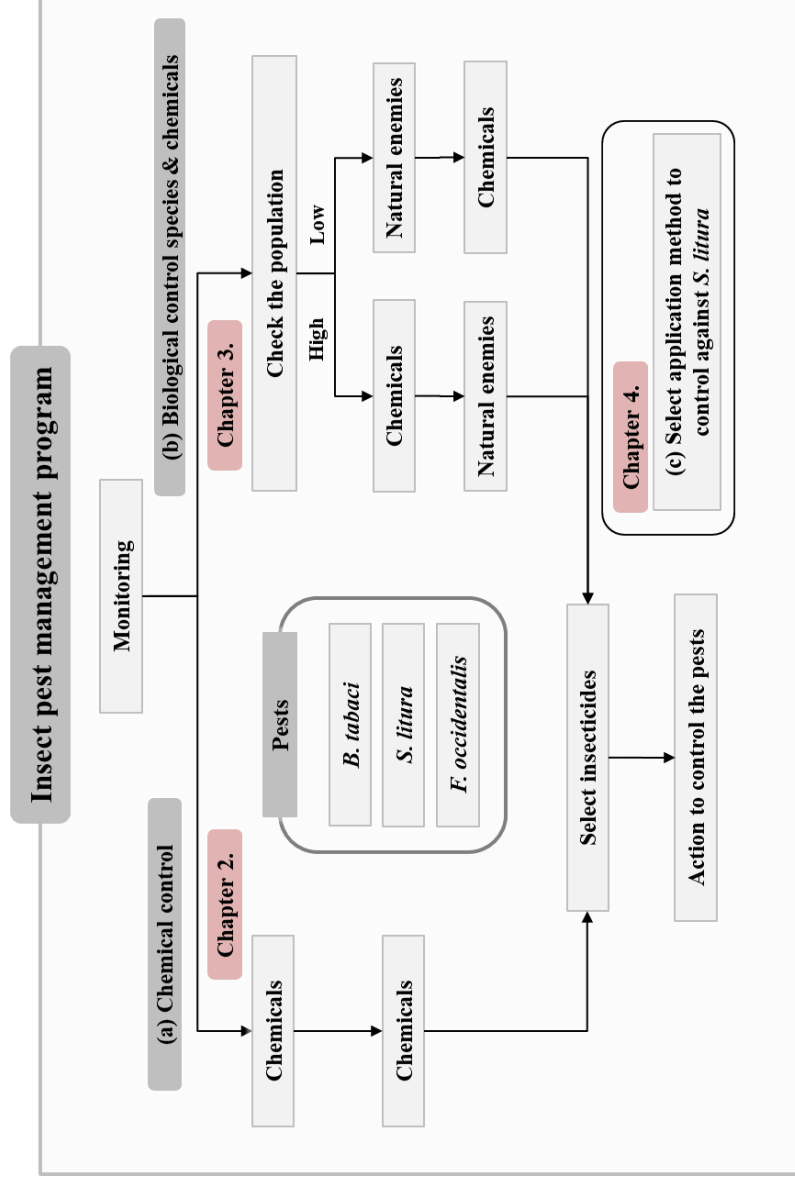


Fig. 1.2. Flow chart of the management strategy in this thesis. There are three main parts: (a) chemical control using insecticides (Chapter 2); (b) pest management with biological control species and insecticides (Chapter 3); and (c) application method for the successful control against *S. litura* with chlorantraniliprole (Chapter 4).

Chapter 2.

**Selection of effective insecticides to control
Bemisia tabaci, *Spodoptera litura*, and
Frankliniella occidentalis in greenhouses**

Abstract

Bemisia tabaci, *S. litura*, and *F. occidentalis* are the main insect pests in ornamental and greenhouse crops and their main control tactic is frequent spraying of various insecticides. To recommend the effective insecticides, with their information on mode of action, the toxicities of 44, 11, and 9 insecticides were evaluated against egg, nymphal, and adult stages of *B. tabaci*, larval stage of *S. litura*, and adult stage of *F. occidentalis*, respectively. Three avermectins (abamectin, emamectin benzoate, and lepimectin), 1 METI acaricide (pridaben), 1 neonicotinoid (dinotefuran), and 1 spinosyn (spinetoram) were selected as the most effective insecticides to all stages of *B. tabaci*. All the insecticides tested were highly toxic to *S. litura* within a day, and 4 insecticides (chlorantraniliprole, cyantraniliprole, flubendiamide and indoxacarb) showed high residual toxicities until 21 days. Spinetoram, spinosad, and emamectin benzoate showed high contact and ingestion toxicities to *F. occidentalis* adults, and chlorfenapyr showed high ingestion toxicity but no contact toxicity to *F. occidentalis* adults. For successful chemical control, 6 insecticides against *B. tabaci*, 10 insecticides against *S. litura*, and 4 insecticides against *F. occidentalis* were selected and the insecticides should be sprayed by rotating chemicals with different mode of action.

Keywords: Insecticides, Toxicity, *Bemisia tabaci*, *Spodoptera litura*,
Frankliniella occidentalis

2.1. Introduction

On many fruit and leaf vegetable in greenhouse cultivation systems, *B. tabaci*, *S. litura*, and *F. occidentalis* are major insect pests in South Korea (Chung, 2001; Park et al., 2002; Park et al., 2010; Lim et al., 2012). *Bemisia tabaci* has high genetic variability, and a species complex of 40 biotypes have been reported over 900 host plants (Perring, 2001; Helmi, 2010; Park, 2012; Khatun et al., 2018). The B biotype is originated in the Middle East and Asian regions, and the Q biotype is originated in the Mediterranean Basin regions, also called as *B. tabaci* Mediterranean (MED) (De Barro et al., 2011, Wang et al., 2016). Mixed occurrence of B and Q biotypes of *B. tabaci* has been reported, but Q biotype has been dominant in greenhouse cultivating environment in South Korea (Table 2.1). The population of B biotype dominated crops grown in open field, while the population of Q biotype gradually dominated crops grown in protected conditions, where resistance outbreaks usually after several insecticide applications (Kontsedalov et al., 2012). Q biotype *B. tabaci* was found to be resistant to neonicotinoid insecticides, whereas B biotype was highly susceptible to them in South Korea (Lee et al., 2012). Spraying insecticides is the primary strategy to control the population of *B. tabaci* and to reduce the damage on the host plant (Sharaf, 1986; Lee et al., 2002). However, the repeated spray

and overuse of insecticides caused the development of resistance to numerous conventional insecticides and made the disruption on the control strategies against *B. tabaci* (Erdogan et al., 2008; Palumbo et al., 2001; Basit et al., 2011; Lee et al., 2012).

Spodoptera litura has been a problem on various fruit and leaf vegetables in South Korea. Lim (2012) investigated that *S. litura* and *Spodoptera exigua* Hübner occurred on five families and 20 species of greenhouse vegetables in Jeonbuk province. Bae (2007) reported the occurrence of both species using sex pheromone traps at different locations and climatic regions in Yeongnam region and *S. litura* adults occurred from mid-March to late November. The densities of *S. litura* were negatively correlated with the yield of leaf vegetables in greenhouse. The densities of 7-10 *S. litura* larvae (2nd~3rd instars) per plant caused about 5% loss of yield in soybean (Lee et al., 2006). Amount of leaves consumed increased with larval age, and larvae consumed 74% of total amount during the last instar stage (Bae, 1999). Fruit damage of sweet pepper was also highly correlated with 2nd instar larval densities of *S. litura* in greenhouse (Park et al., 2010). Farmers primarily rely on the use of insecticides to reduce crop losses due to *S. litura*. The lethal concentration (LC₅₀) dose of insecticides was rapidly increased with larval age of *S. litura* from 0.5ppm (1st instar) to 546.6ppm

(4th instar) (Bae et al., 2003). The insecticide resistance of *S. litura* has been reported in the world. Ahmad (2007) reported that the field populations of *S. litura* from Pakistan showed a positive cross-resistance within the same class such as organophosphates, carbamates, or pyrethroids, but there was no cross-resistance among the different classes. The resistance ratios of *S. litura* were from 8 to 162 folds for organophosphates and pyrethroids, but for new insecticides such as spinosad, indoxacarb, lufenuron, and methoxyfenozide, resistance levels were 2-74 folds and lower than organophosphates and pyrethroids (Saleem et al., 2016). Muthusamy (2014) reported the development of resistance up to 80 folds against a new insecticide, chlorantraniliprole, which was registered recently in India. To avoid the development of resistance, various insecticides with different mode of action should be sprayed (Ahmad et al., 2007).

The seasonal occurrence of *F. occidentalis* showed the first peak between mid-June and late-July and the second peak between mid-August and mid-October in environmentally friendly lettuce greenhouses (Jeon et al., 2006). The highest densities were observed from late-April to mid-June and from mid-August to early September in Chrysanthemum greenhouses (Park et al., 2002). *Frankliniella occidentalis* adult female preferred tomato to eggplant, cucumber, and red pepper. Among four plants tested, the most

preferred part was petals of red pepper. *Frankliniella occidentalis* adult female fed preferably on petal compared with leaves (Lee et al., 2003). The niche of thrips has four phases: eggs within plant tissue, young larvae on the plant surface, old larvae and pupae in soil, and adults deep inside the flower and in the air (Chung, 2001). Farmers are spraying insecticides to control *F. occidentalis*, but its control is so difficult due to the different niche for each stage.

Consequently, sustainable management strategies against the major pests such as *B. tabaci*, *S. litura*, and *F. occidentalis* in greenhouse cultivating systems have been dependent on the availability of conventional insecticides and their long-lasting efficacy. In this study, the effective insecticides against these insect pests were selected by a bioassay with their information on the mode of action.

Table 2.1. The occurrence of B & Q biotype *B. tabaci* in South Korea.

Year	Biotype	Occurrence place	Note	Reference
2005	Q	Chungbuk, Chungnam, Gyongnam, Jeonnam	First founding of the Q biotype in South Korea.	Lee et al., 2005
	B	Chungbuk		
2012	B & Q	Gyeonggi	Widespread throughout the southern part. The population was mixed by the two biotypes. Q biotype was resistant to neonicotinoid insecticides, whereas biotype B was highly susceptible.	Lee et al., 2012
	Q	Gyeongbuk, Gyeongnam, Daegu, Daejeon, Kwangju, Jeonbuk, Jeonnam, Chungnam, Jeju		
	B	Gyeonggi		
2014	Q	Gyeongbuk	First report of occurrence in oriental melon with mixed infection by two biotypes. Q biotype was investigated in 11 greenhouses and B biotype in 4 greenhouses	Kim and Kim, 2014
	B			

2.2. Materials and methods

2.2.1. Insect colony

Bemisia tabaci (Q biotype) and *S. litura* obtained from Chungbuk National University in 2005 were reared on red pepper, *Capsicum annuum*, under standard laboratory condition of $25 \pm 1^\circ\text{C}$, a photoperiod of 16:8 (L:D) h, and 65% RH. *Frankliniella occidentalis* was collected from cucumber greenhouse in Daejeon in 2006 and reared on soybean plants under the same environment condition as described above. During rearing in the laboratory, *B. tabaci*, *S. litura*, and *F. occidentalis* were isolated and not treated with any insecticides.

2.2.2. Toxicity assay against *B. tabaci*

Insecticides used in this experiment were summarized in Table 2.2. Each insecticide solution was made by diluting insecticides with 100 ml of distilled water to make field recommended concentration. For this experiment, 25 adult females (< 5 days old) were placed in an insect breeding square dish (dimension $80.8 \times 80.8 \times 21.0$ mm) for 48 hours to obtain the same stage of egg and 2nd instar for the bioassays. All the

experiments were replicated by three times in the standard laboratory condition above.

Leaf disc (50 mm diameter) infested with 11-110 eggs (2 days old) were dipped in various insecticide solutions for 30 seconds and in distilled water as a control. After application, the leaves were dried for 1 hour and placed on the insect breeding dish (dimension 100 × 41 mm). Cumulative egg mortality was determined by checking the nymphs at 10 days after application.

Pepper plants (80 mm height) infested with 2nd instars (average 53 nymphs per replication) were sprayed with 1 ml of insecticide solution and distilled water as a control using a hand sprayer (5 ml cap.) and dried for 1 hour. The plants were placed in the insect breeding square dish and in the standard laboratory condition above. Death of nymphs was checked at 7 days, and emergence of adults was checked at 11 days after application. Death was confirmed when the nymphs did not molt into 4th instar or did not emerge to adults.

To evaluate the toxicity to adults, 25 adult females (< 5 days old) were placed in an insect breeding square dish. Insecticides were sprayed and dried with the same method as described above. Death of adults was

checked at 2 days after application. Death was confirmed when adults did not move 1.5 times of length of their body when touched with a fine brush.

2.2.3. Toxicity assay against *S. litura*

Insecticides used in this experiment were summarized in Table 2.2. Insecticide solutions were prepared by the same methods as in the toxicity test against *B. tabaci*. Treatment was made by spraying insecticides at the recommended dose to red pepper plants (25 cm height).

Leaf discs (50 mm diameter) of the red peppers were collected at 0, 7, 14, 21, 48 days after application and placed each in the insect breeding dish. Twenty 2nd instar larvae (< 2 days old) were placed on each leaf disc and death of larvae was checked after 4 days. Death was confirmed when larvae did not move 1.5 times of length of their body when touched with a fine brush. All the experiments were replicated by three times and conducted in the standard laboratory condition described above.

2.2.4. Toxicity assay against *F. occidentalis*

Insecticides used in this experiment were summarized in Table 2.2. Insecticides solution was made by the same methods as in the toxicity test against *B. tabaci*.

2.2.4.1. Contact toxicity assay

Twenty-five adults (< 5 days old) of *F. occidentalis* were dipped individually for 30 seconds in each insecticide solution and placed in an insect breeding dish for 2 days with leaf disc (50 mm diameter) of soybean which was not treated with any insecticide. Death was confirmed when adults did not move 1.5 times of length of their body when touched with a fine brush. All the experiments were replicated by three times and conducted in the standard laboratory condition described above.

2.2.4.2. Ingestion toxicity assay

Leaf discs (50 mm diameter) of soybean plants were dipped for 30 seconds in each insecticide solution and dried for 1 hour. The leaf discs were placed in an insect breeding dish with wetting filter paper (90 mm diameter). Twenty-five adults *F. occidentalis* (< 5 days old) were placed in the insect breeding dish for 2 days. Death was confirmed when adults did

not move 1.5 times of length of their body when touched with a fine brush. All the experiments were replicated by three times and conducted in the standard laboratory condition described above.

2.2.5. Data analysis

Corrected mortality percentage was calculated using Abbott's formula (Abbott, 1925):

$$\% \text{ corrected mortality} = \frac{X - Y}{X} \times 100$$

Where X = percentage survivorship in the control and Y = percentage survivorship in the treatment.

Percentage mortalities were arcsine-transformed before statistical analysis. A one-way analysis of variance (ANOVA) was conducted, followed by Tukey multiple-range tests at $P < 0.05$ for mean separation, using PROC GLM in SAS (SAS Institute, 2009).

Table 2.2. Lists of insecticides used in toxicity assay.

Common name	Target pests	Chemical group (IRAC MoA No.)	Formulation	AI (%)	Recommended conc.(ppm)	Pests for assay	
Abamectin	Mites, Leaf miners, Colorado beetles	Avermectins	(6)	EC	1.8	6	<i>B. tabaci</i>
Emamectin benzoate	Lepidopterans	Avermectins	(6)	EC	2.15	10.75	<i>B. tabaci</i> , <i>S. litura</i> , <i>F. occidentalis</i>
Lepimectin	Mites, Leaf miners, Colorado beetles	Avermectins	(6)	EC	2	10	<i>B. tabaci</i>
Bistrifluron	Lepidopterans, Whiteflies	Benzoylureas	(15)	EC	10	50	<i>B. tabaci</i> , <i>S. litura</i>
Flufenoxuron	Lepidopterans, mites	Benzoylureas	(15)	DC	5	50	<i>B. tabaci</i>
Lufenuron	Lepidopterans	Benzoylureas	(15)	EC	5	25	<i>B. tabaci</i>
Teflubenzuron	Lepidopterans	Benzoylureas	(15)	SC	5	50	<i>B. tabaci</i>
Bifenazate		Bifenazate	(20D)	SC	23.5	117.5	<i>B. tabaci</i>
Carbaryl		carbamates	(1A)	WP	50	500	<i>B. tabaci</i>
Fenobucarb	Lepidopterans	carbamates	(1A)	EC	50	500	<i>B. tabaci</i>
Chlorfenapyr	Lepidopterans, Mites, Thrips	Chlorfenapyr	(13)	SC	10	50	<i>B. tabaci</i> , <i>S. litura</i> , <i>F. occidentalis</i>
Methoxyfenozide	Lepidopterans	Diacylhydrazines	(18)	SC	21	105	<i>B. tabaci</i> , <i>S. litura</i>
Chlorantraniliprole	Lepidopterans	Diamides	(28)	WG	5	25	<i>B. tabaci</i> , <i>S. litura</i>

Table 2.2. *Continued.*

Cytraniliprole	Lepidopterans	Diamides	(28)	OD	10	50	<i>B. tabaci</i> , <i>S. litura</i>
Flubendiamide	Lepidopterans	Diamides	(28)	WG	20	100	<i>B. tabaci</i> , <i>S. litura</i>
Etoxazole	Mites	Etoazole	(10B)	SC	10	25	<i>B. tabaci</i>
Flonicamid	Sucking insects	Flonicamid	(29)	WG	10	50	<i>B. tabaci</i>
Indoxacarb	Lepidopterans	Indoxacarb	(22A)	SC	5	50	<i>B. tabaci</i> , <i>S. litura</i>
Fenazaquin	Mites	METI acaricides	(21A)	SC	20	67	<i>B. tabaci</i>
Fenpyroximate	Mites	METI acaricides	(21A)	SC	5	250	<i>B. tabaci</i>
Pyridaben	Whiteflies, Mites	METI acaricides	(21A)	SC	20	133	<i>B. tabaci</i>
Acetamiprid	Sucking insects	Neonicotinoids	(4A)	WP	8	40	<i>B. tabaci</i> , <i>F. occidentalis</i>
Clothianidin	Sucking insects	Neonicotinoids	(4A)	SC	8	40	<i>B. tabaci</i>
Dinotefuran	Sucking insects	Neonicotinoids	(4A)	WP	10	100	<i>B. tabaci</i> , <i>F. occidentalis</i>
Imidacloprid	Sucking insects	Neonicotinoids	(4A)	WP	10	50	<i>B. tabaci</i> , <i>F. occidentalis</i>
Thiacloprid	Sucking insects	Neonicotinoids	(4A)	SC	10	50	<i>B. tabaci</i>
Thiamethoxam	Sucking insects	Neonicotinoids	(4A)	WG	10	50	<i>B. tabaci</i>
Sulfoxaflor	Sucking insects	Sulfoxaflor	(4C)	SC	7	35	<i>F. occidentalis</i>
Chlorpyrifos	Lepidopterans	Organophosphates	(1B)	WP	25	250	<i>B. tabaci</i> , <i>S. litura</i>
Chlorpyrifos-methyl	Lepidopterans	Organophosphates	(1B)	CS	25	250	<i>B. tabaci</i>
Fenitrothion	Lepidopterans	Organophosphates	(1B)	WP	40	400	<i>B. tabaci</i>
Prothiofos	Lepidopterans	Organophosphates	(1B)	EC	50	500	<i>B. tabaci</i>
Azocyclotin	mites	Organotin miticides	(12B)	WP	25	162.5	<i>B. tabaci</i>

Table 2.2. *Continued.*

Acrinathrin	Thrips	Pyrethroids	(3A)	WP	5.7	57	<i>B. tabaci</i> , <i>F. occidentalis</i>
Bifenthrin	Lepidopterans	Pyrethroids	(3A)	WG	8	20	<i>B. tabaci</i> , <i>S. litura</i>
Cyfluthrin	Lepidopterans	Pyrethroids	(3A)	WP	5	25	<i>B. tabaci</i>
Etofenprox	Lepidopterans	Pyrethroids	(3A)	WP	10	100	<i>B. tabaci</i>
Fenvalerate	Lepidopterans	Pyrethroids	(3A)	EC	5	50	<i>B. tabaci</i>
Gamma-cyhalothrin	Lepidopterans	Pyrethroids	(3A)	CS	1.4	5.6	<i>B. tabaci</i>
Pymetrozine	Sucking insects	Pyridine azomethine derivatives	(9B)	WP	25	83.75	<i>B. tabaci</i>
Pyriproxyfen	Whiteflies	Pyriproxyfen	(7C)	EC	10	100	<i>B. tabaci</i>
Spinetoram	Lepidopterans, Thrips, Flies, Beetles, Grasshoppers	Spinosyns	(5)	WG	5	25	<i>B. tabaci</i> , <i>S. litura</i> , <i>F. occidentalis</i>
Spinosad	Lepidopterans, Thrips, Flies, Beetles, Grasshoppers	Spinosyns	(5)	SC	10	50	<i>B. tabaci</i> , <i>F. occidentalis</i>
Spirodiclofen	Mites	Tetronic and Tetramic acid derivatives	(23)	WP	36	180	<i>B. tabaci</i>
Spiromesifen	Mites	Tetronic and Tetramic acid derivatives	(23)	SC	20	100	<i>B. tabaci</i>

2.3. Results

2.3.1. Toxicity assay against *B. tabaci*

Eggs, nymphs, and adults of *B. tabaci* showed different susceptibility to insecticides tested. Against eggs, abamectin, emamectin benzoate, lepimectin, cyantraniliprole, fenpyroximate, pyridaben, dinotefuran, imidacloprid, thiacloprid, spinetoram, spinosad, and spiromesifen were most effective, causing 100% mortality, and fenazaquin, acetamiprid, thiamethoxam, chlorpyrifos, and prothiofos were also highly effective, causing > 90% mortality (Table 2.3) ($F = 12.65$; $df = 43, 82$; $P < 0.001$). Against nymphs, abamectin, emamectin benzoate, lepimectin, cyantraniliprole, fenpyroximate, pyridaben, dinotefuran, and spiromesifen were effective (Table 2.4) ($F = 17.76$; $df = 43, 88$; $P < 0.001$). Abamectin, emamectin benzoate, lepimectin, dinotefuran, pyridaben, azocyclotin, and spinetoram were effective against adults (Table 2.5) ($F = 18.82$; $df = 43, 88$; $P < 0.001$).

Table 2.3. Toxicity of insecticides against *B. tabaci* eggs at 10 days after application.

Treatments	Percent corrected mortality (Mean \pm SD)			
	Mean		STDEV	
Abamectin	100.0			a
Emamectin benzoate	100.0			a
Lepimectin	100.0			a
Bistrifluron	65.3	\pm 10.3		abcdefg
Flufenoxuron	18.7	\pm 3.9		fghi
Lufenuron	67.9	\pm 12.5		abcdefg
Teflubenzuron	34.2	\pm 41.8		cdefghi
Bifenazate	2.1	\pm 1.8		i
Carbaryl	50.3	\pm 28.7		abcdefghi
Fenobucarb	6.8	\pm 12.3		ghi
Chlorfenapyr	87.2	\pm 0.7		abcd
Methoxyfenozide	20.4	\pm 16.7		fghi
Chlorantraniliprole	40.4	\pm 17.7		abcdefghi
Cyantraniliprole	100.0			a
Flubendiamide	9.4	\pm 18.8		fghi
Etoxazole	32.0	\pm 16.3		defghi
Flonicamid	61.7	\pm 16.5		abcdefghi
Indoxacarb	50.3	\pm 11.7		abcdefghi
Fenazaquin	96.3	\pm 7.3		ab
Fenpyroximate	100.0			a
Pyridaben	100.0			a
Acetamiprid	91.2	\pm 13.2		abcd
Clothianidin	68.6	\pm 27.3		abcdef

Table 2.3. *Continued.*

Dinotefuran	100.0		a
Imidacloprid	100.0		a
Thiacloprid	100.0		a
Thiamethoxam	98.2	± 2.5	ab
Chlorpyrifos	94.5	± 0.7	abc
Chlorpyrifos-methyl	64.6	± 30.6	abcdef
Fenitrothion	22.1	± 24.2	fghi
Prothiofos	97.5	± 5.1	ab
Azocyclotin	25.6	± 22.3	efghi
Acrinathrin	23.5	± 27.8	efghi
Bifenthrin	69.0	± 4.4	abcdef
Cyfluthrin	46.6	± 0.3	abcdefghi
Etofenprox	63.8	± 5.8	abcdefgh
Fenvalerate	3.4	± 4.0	hi
Gamma-cyhalothrin	45.9	± 5.1	abcdefghi
Pymetrozine	37.0	± 32.3	bcdefghi
Pyriproxyfen	84.0	± 2.0	abcde
Spinetoram	100.0		a
Spinosad	100.0		a
Spirodiclofen	21.9	± 18.3	fghi
Spiromesifen	100.0		a

Table 2.4. Toxicity of insecticides against *B. tabaci* nymphs at 7 and 11 days after application.

Treatments	Percent corrected mortality (Mean \pm SD)			
	at 7 days after application		at 11 days after application	
	Mean	STDEV	Mean	STDEV
Abamectin	88.6 \pm 10.4	ab	93.4 \pm 2.5	abc
Emamectin benzoate	92.3 \pm 5.8	a	89.6 \pm 7.7	abc
Lepimectin	91.0 \pm 8.5	a	93.9 \pm 5.8	abc
Bistrifluron	69.1 \pm 10.8	abcdef	50.4 \pm 11.6	cdefghij
Flufenoxuron	74.9 \pm 10.9	abcd	75.1 \pm 17.3	abcdef
Lufenuron	10.5 \pm 10.5	hij	6.6 \pm 11.5	jk
Teflubenzuron	20.8 \pm 7.9	fghij	18.3 \pm 10.0	ijk
Bifenazate	28.5 \pm 7.4	cdefghij	27.4 \pm 15.3	fghijk
Carbaryl	11.2 \pm 19.4	hij	0.0	k
Fenobucarb	37.9 \pm 20.2	bcdefghij	41.1 \pm 13.1	defghijk
Chlorfenapyr	16.5 \pm 28.6	ghij	22.7 \pm 21.5	hijk
Methoxyfenozide	25.6 \pm 12.1	efghij	15.5 \pm 12.9	ijk
Chlorantraniliprole	46.2 \pm 42.0	abcdefghij	0.7 \pm 1.2	k
Cyantraniliprole	96.3 \pm 1.6	a	96.9 \pm 2.7	ab

Table 2.4. *Continued.*

Flubendiamide	13.1 ± 9.2	hij	25.5 ± 11.1	ghijk
Etazazole	28.8 ± 19.7	cdefghij	29.1 ± 23.6	fghijk
Flonicamid	94.8 ± 1.6	a	82.0 ± 13.4	abcd
Indoxacarb	17.6 ± 17.5	fghij	11.4 ± 19.7	jk
Fenazaquin	89.8 ± 2.1	ab	88.5 ± 3.6	abcd
Fenpyroximate	80.5 ± 5.2	abc	90.6 ± 4.1	abc
Pyridaben	93.3 ± 3.9	a	96.1 ± 3.5	ab
Acetamiprid	69.8 ± 14.9	abcdef	67.2 ± 19.0	abcdefgh
Clothianidin	56.2 ± 9.5	abcdefghij	51.9 ± 13.5	bcdefghi
Dinotefuran	95.7 ± 2.1	a	95.9 ± 2.5	ab
Imidacloprid	20.0 ± 33.3	fghij	33.9 ± 44.0	fghijk
Thiacloprid	88.2 ± 10.6	ab	88.9 ± 9.4	abcd
Thiamethoxam	73.1 ± 13.6	abcde	79.9 ± 14.7	abcde
Chlorpyrifos	75.9 ± 9.3	abcd	13.2 ± 22.9	jk
Chlorpyrifos-methyl	68.2 ± 15.2	abcdefg	66.5 ± 14.1	abcdefgh
Fenitrothion	67.3 ± 16.5	abcdefg	62.3 ± 11.9	abcdefghi
Prothiofos	89.6 ± 2.8	ab	87.3 ± 3.4	abcd

Table 2.4. *Continued.*

Azocyclotin	81.9	±	4.0	ab	83.3	±	4.3	abcd
Acrinathrin	73.5	±	20.8	abcde	72.2	±	28.5	abcdefg
Bifenthrin	8.9	±	10.1	hij	0.0			k
Cyfluthrin	60.7	±	26.0	abcdefg	7.2	±	7.0	jk
Etofenprox	27.0	±	37.3	efghij	0.0			k
Fenvalerate	58.3	±	14.7	abcdefghij	61.5	±	17.7	abcdefghi
Gamma-cyhalothrin	5.9	±	10.2	j	0.0			k
Pymetrozine	37.6	±	10.0	bcdefghij	47.7	±	13.0	cdefghijk
Pyriproxyfen	7.2	±	10.0	ij	78.2	±	20.4	abcd
Spinetoram	88.2	±	10.8	ab	89.7	±	6.2	abc
Spinosad	59.1	±	11.6	abcdefghi	82.6	±	16.8	abcd
Spirodiclofen	17.8	±	17.9	fghij	21.5	±	17.1	hijk
Spiromesifen	95.4	±	5.0	a	100.0			a

Table 2.5. Toxicity of insecticides against *B. tabaci* adult females at 2 days after application.

Treatments	Percent corrected mortality (Mean \pm SD)			
	Mean		STDEV	
Abamectin	90.5	\pm 10.2		ab
Emamectin benzoate	80.3	\pm 4.9		abcd
Lepimectin	98.6	\pm 2.3		ab
Bistrifluron	13.5	\pm 9.4		hij
Flufenoxuron	6.6	\pm 4.9		ij
Lufenuron	6.6	\pm 4.9		ij
Teflubenzuron	0.5	\pm 1.0		j
Bifenazate	7.7	\pm 8.3		ij
Carbaryl	4.5	\pm 5.7		ij
Fenobucarb	19.1	\pm 20.7		fghij
Chlorfenapyr	44.6	\pm 31.0		cdefghi
Methoxyfenozide	4.9	\pm 2.8		ij
Chlorantraniliprole	0.9	\pm 1.6		j
Cyantraniliprole	19.7	\pm 17.3		fghij
Flubendiamide	14.2	\pm 13.3		hij
Etoxazole	12.0	\pm 20.9		ij
Flonicamid	75.7	\pm 14.6		abcd
Indoxacarb	75.7	\pm 14.6		abcd
Fenazaquin	67.2	\pm 2.8		abcde
Fenpyroximate	6.0	\pm 9.0		ij
Pyridaben	86.9	\pm 7.5		abc
Acetamiprid	27.9	\pm 15.8		fghij
Clothianidin	24.6	\pm 7.5		fghij

Table 2.5. *Continued.*

Dinotefuran	98.6	±	2.3	ab
Imidacloprid	60.8	±	10.2	abcdef
Thiacloprid	55.7	±	9.8	bcdefg
Thiamethoxam	12.6	±	16.4	ij
Chlorpyrifos	6.3	±	10.9	ij
Chlorpyrifos-methyl	25.7	±	22.4	fghij
Fenitrothion	27.9	±	7.5	fghij
Prothiofos	57.4	±	10.2	abcdefg
Azocyclotin	91.8	±	7.5	ab
Acrinathrin	15.8	±	13.9	ghij
Bifenthrin	39.2	±	0.0	fghij
Cyfluthrin	24.8	±	25.7	fghij
Etofenprox	20.7	±	19.7	fghij
Fenvalerate	6.0	±	9.0	ij
Gamma-cyhalothrin	19.4	±	19.6	fghij
Pymetrozine	6.0	±	5.8	ij
Pyriproxyfen	3.6	±	6.2	ij
Spinetoram	100.0			a
Spinosad	64.9	±	19.2	abcde
Spirodiclofen	24.6	±	7.5	fghij
Spiromesifen	25.7	±	16.4	fghij

2.3.2. Toxicity assay against *S. litura*

All the insecticides tested were highly toxic to *S. litura* within a day of the applications (Fig. 2.1). After 7 days, the mortality by emamectin benzoate decreased rapidly, and it showed the lowest residual activity among the insecticides tested. The toxicities of bistrifluron, chlorfenapyr, methoxyfenozide, chlorpyrifos, bifenthrin and spinetoram lasted for 7 days, but didn't last for 14 days. Four insecticides (Chlorantraniliprole, cyantraniliprole, flubendiamide, and indoxacarb) showed high residual activities until 21 days. At 48 days, the toxicity of indoxacarb decreased rapidly, and cyantraniliprole and flubendiamide showed 58% and 62% mortalities, respectively. The toxicity of chlorantraniliprole lasted longest, and the mortality was 78% at 48 days.

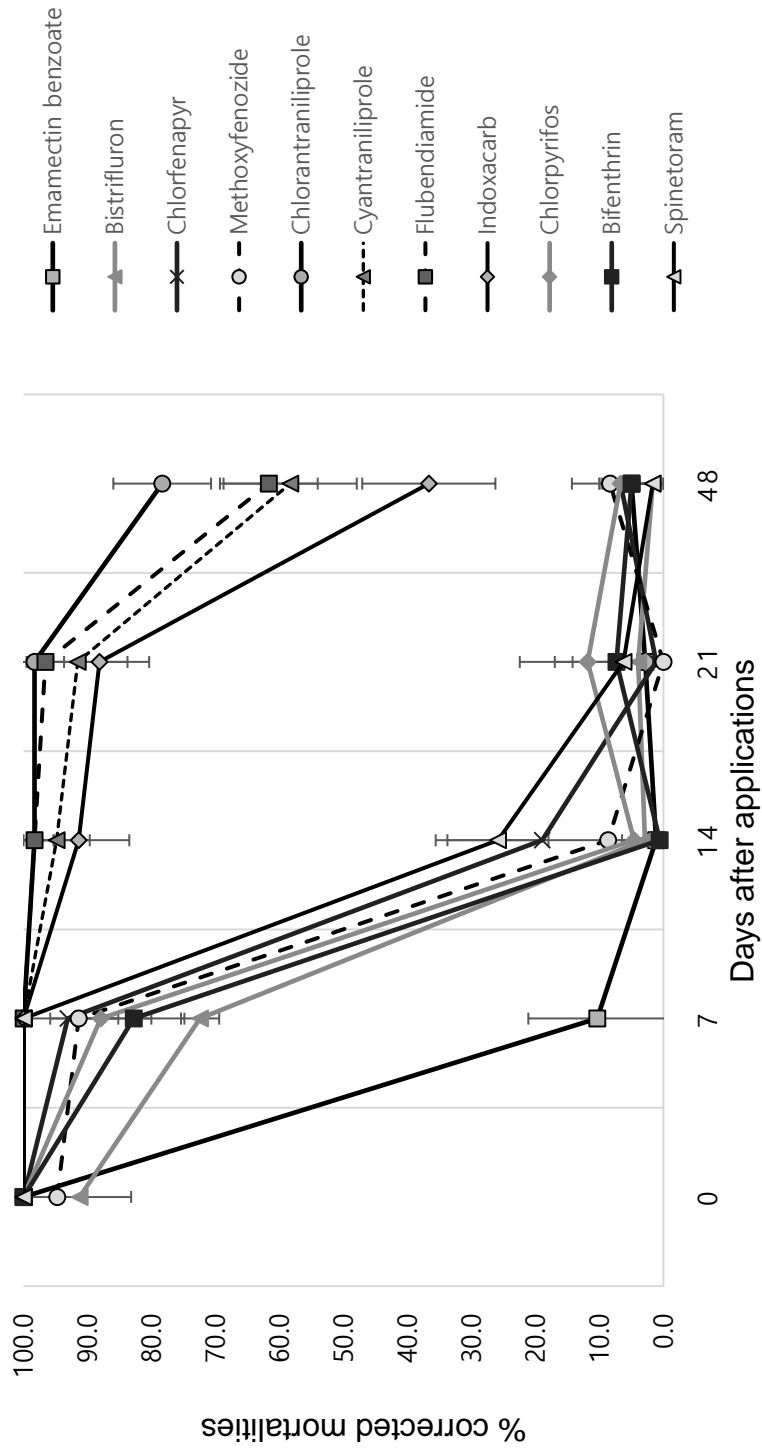


Fig. 2.1. Toxicity of insecticides against *S. litura* 2nd instar stages at 0, 7, 14, 21, and 48 days after application

2.3.3. Toxicity assay against *F. occidentalis*

Spinetoram, spinosad, and emamectin benzoate showed high contact toxicities to *F. occidentalis* adult stages (Fig. 2.2). The contact toxicities of spinetoram and spinosad were found at 3 hours after application ($F = 55.02$; $df = 8, 18$; $P < 0.001$). The mortality of emamectin benzoate was 68.0% at 3 hours and reached 98.7% at one day after application ($F = 631.89$; $df = 8, 18$; $P < 0.001$). Spinetoram, spinosad, chlorfenapyr, and emamectin benzoate showed 100% ingestion toxicities to *F. occidentalis* adult stages at 2 days ($F = 1654.31$; $df = 8, 18$, $P < 0.001$) (Fig. 2.3).

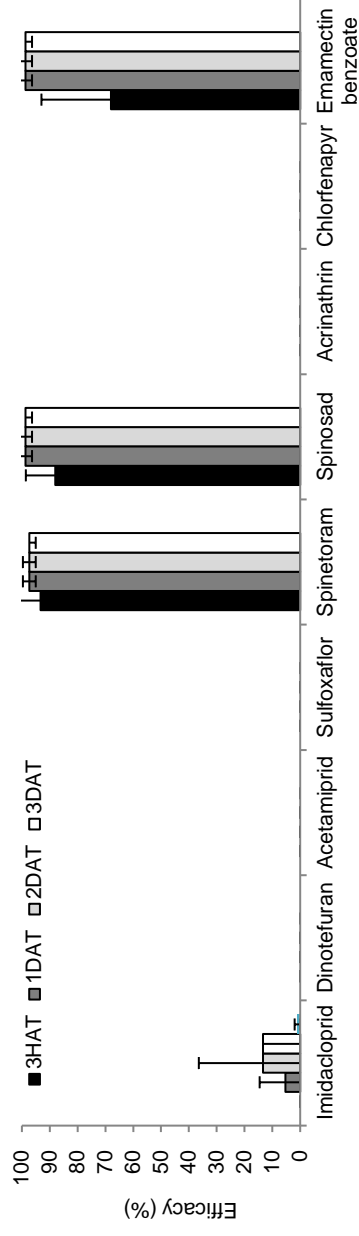


Fig. 2.2. Contact toxicity of insecticides against *F. occidentalis* adult stages.

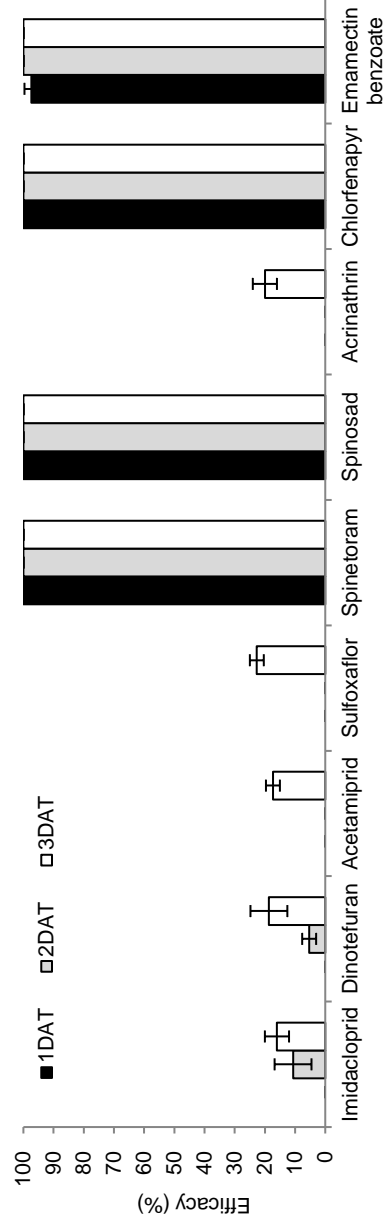


Fig. 2.3. Ingestion toxicity of insecticides against *F. occidentalis* adult stages.

2.4. Discussion

2.4.1. Toxicity test against *B. tabaci*

In this study, all the avermectins and METI acaricides tested were highly toxic to all the life stages of *B. tabaci*. Ma (2007) showed very similar results that resistant strain of *B. tabaci* to pyrethroids was not resistant to abamectin. Cuthbertson (2012) also found that abamectin and acetamiprid were excellent ovicidal agents of *B. tabaci*. Cyantraniliprole of diamide insecticides will be an effective control agent for control of *B. tabaci* due to high toxicities to egg and nymph stages (Carmeron et al., 2016). Caballero (2013) also suggested that cyantraniliprole will be an essential component of a resistance management program due to the high susceptibility and no resistance between laboratory and field colonies of *B. tabaci* B biotype in southern Florida. Neonicotinoids except for clothianidin showed high toxicities to egg stages, but dinotefuran was also toxic to nymph and adult stages. Therefore, dinotefuran was an alternative insecticide to control all the stage of *B. tabaci* in this study. Byrne (2010) also proved the high toxicities of dinotefuran to *B. tabaci* in foliar contact and soil drench applications. If dinotefuran is used repeatedly in a growing season, resistance and cross-resistance of *B. tabaci* among neonicotinoid

insecticides may increase rapidly. Basit (2011) proved that, after selection for eight generations with acetamiprid which is the same chemical group with dinotefuran, resistance increased rapidly from 3 to 118-fold, and cross-resistance was also verified in imidacloprid, thiamethoxam, thiacloprid, nitenpyram, endosulfan, and bifenthrin. *Bemisia tabaci* egg and nymph were highly susceptible to spiromesifen, tetronic and tetramic acid derivatives, but adult stage was less susceptible to the insecticide. Liu (2004) also proved that residues of spiromesifen were not toxic to *B. tabaci* adults.

In this study, METI acaricides are proved as effective insecticides against egg and nymph stage, but showed low toxicity to adults except pyridaben. Spinosyns and two organophosphates (chlorpyrifos and prothiofos) also showed high toxicities to egg stages, but only spinetoram showed toxicities to nymph and adult stages. Azocyclotin, organotin miticides showed high toxicity to adult stages by causing 91.8% mortalities. Three avermectins (abamectin, emamectin benzoate, and lepimectin), pyridaben, dinotefuran, and spinetoram can be used in rotation to control all life stages of *B. tabaci*. Cyantraniliprole, 2 METI acaricides (fenazaquin and fenpyroximate), and spiromesifen may be useful as alternative insecticides to control the population and reduce the insecticide resistance due to the high toxicities to egg and nymph stages of *B. tabaci*. But, efficacies of

selected insecticides against Q biotype *B. tabaci* in this study might be overestimated due to existence of Insecticide resistant populations of *B. tabaci* in either fields or greenhouse where insecticides had been treated.

2.4.2. Toxicity test against *S. litura*

All the insecticides tested showed high toxicity to *S. litura* within a day of the applications (Fig. 2.1). El-Sheikh (2015) suggested that emamectin benzoate was an effective compound compared with lufenuron and spinosad for the control of *Spodoptera littoralis* Boisd. However, the mortality of emamectin benzoate rapidly decreased at 7 days which may be due to the dissipation of emamectin benzoate with half-life ($t_{1/2}$) of 1.0-1.3 days (Zhou et al., 2016). In this study, although the residual efficacy of insecticides varied, all the insecticides tested showed high toxicity to *S. litura* and can be used as alternative control agents. But, the occurrence of insecticide resistance in *S. litura* has been reported and resulted in control failure in field. Saleem (2016) found high resistance to organophosphates and pyrethroids and suggested to stop using organophosphates against *S. litura*. Ahmad (2007) found the positive correlation of resistance within the same class, but there was no cross-resistance among different classes of insecticides. Three diamide insecticides (chlorantraniliprole, cyantraniliprole,

and flubendiamide) with characteristics of lower degradation in crops showed residual toxicities until 48 days and were the most effective control agents against *S. litura* in this study (EPA, 2018). But, the resistance of *S. litura* against chlorantraniliprole has been reported in fields (Su et al., 2012; Muthusamy et al., 2014). To reduce the occurrence of resistance and to use the insecticides effectively, insecticides of diverse mode of action should be sprayed, and the insecticides in the same class should not be used for five generations or more of *S. litura*. The resistance can be reduced if there is no exposure to insecticides (Rehan and Freed, 2014).

2.4.3. Toxicity test against *F. occidentalis*

Spinetoram, spinosad, chlorfenapyr, and emamectin benzoate were effective insecticides for controlling *F. occidentalis*. Seal (2006) suggested with very similar results that chlorfenapyr was the most effective in controlling adults and larvae of *Scirtothrips dorsalis* Hood followed by spinosad and imidacloprid. Kwon (2015) also reported that chlorfenapyr is a better option compared with the other insecticides for the control of *F. occidentalis* in five field populations, suggesting varying degrees of resistance to acrinathrin, thiamethoxam, spinosad, and emamectin benzoate. In our study, however, spinetoram, spinosad, and emamectin

benzoate were found to have high contact and ingestion toxicities. The reason for this discrepancy might be caused by genetic variation of *F. occidentalis* populations tested. The contact and ingestion toxicities of spinetoram and spinosad, fermentation insecticides derived from the actinomycete *Saccharopolyspora spinosa*, occur by disrupting the GABA-gated chloride channels (Depalo et al., 2016). But, due to the biological characteristics of thrips, having separated ecological niche among life stages and the occurrence of resistant population of *F. occidentalis*, farmers have experienced a control failure (Chung, 2001). To control the population of *F. occidentalis*, the effective insecticides with different mode of action should be sprayed in rotation. In this study, we suggest the effective insecticides of different mode of action such as two spinosyns (spinetoram and spinosad), chlorfenapyr, and emamectin benzoate.

Chapter 3.

Selective toxicities of 13 insecticides against seven commercialized biological control species

Abstract

To determine selective insecticides for biological control based IPM, the relative acute toxicities of 13 insecticides against 2 predatory mites (*Phytoseiulus persimilis* Athias-Henriot and *Amblyseius swirskii* Athias-Henriot), 2 hemipteran predators (*Orius laevigatus* [Fieber] and *Nesidiocoris tenuis* Reuter), and 3 hymenopteran parasitoids (*Diglyphus isaea* [Walker], *Aphidius colemani* Viereck, and *Encarsia formosa* Gahan) were compared in direct and residual applications in the laboratory condition. Among them, six insecticides (dinotefuran, indoxacarb, chlorantraniliprole, cyantraniliprole, methoxyfenozide, and bistrifluron) were safe to predatory mites while six insecticides (spinetoram, spinosad, lepimectin, emamectin benzoate, chlorfenapyr, and pyridaben) showed high toxicity to predatory mites in the direct application assay. Flubendiamide was slightly harmful to *P. persimilis* but safe to *A. swirskii*. Bistrifluron, methoxyfenozide, and chlorantraniliprole were not toxic to both hemipteran predators. Most insecticides tested were highly toxic to all hymenopteran parasitoids except *D. isaea* which showed low susceptibility to chlorantraniliprole and flubendiamide. Most insecticides had low residual toxicity to predatory mites except chlorfenapyr of which toxicity was maintained over 7 days to *P. persimilis*. Nine insecticides (bistrifluron, chlorfenapyr, chlorantraniliprole,

cyantraniliprole, emamectin benzoate, flubendiamide, lepimectin, and methoxyfenozide) were safe in 4 days to hemipteran predators. Only cyantraniliprole showed low residual toxicity to *E. formosa* at one day after application, but six insecticides (indoxacarb, chlorantraniliprole, cyantraniliprole, flubendiamide, methoxyfenozide, and bistrifluron) were safe to *D. isaea* and methoxyfenozide and bistrifluron to *A. colemani*.

Keywords: selective insecticide, predatory mite, hemipteran predator, hymenopteran parasitoid

3.1. Introduction

Biological control is an important tactic for integrated pest management (IPM) programs. Numerous biological control species including *P. persimilis*, *E. formosa*, *Eretmocerus eremicus* Rose and Zolnerowich, *Eretmocerus mundus* Mercet, *A. swirskii*, *A. colemani*, and *O. laevigatus* have been used or evaluated for the control of various pests such as *Tetranychus urticae* Koch, *Trialeurodes vaporariorum* Westwood, *Bemisia tabaci* Gennadius, *Aphis gossypii* Glover, and *Frankliniella occidentalis* Pergande in the greenhouse cultivation in South Korea (Ahn et al., 2004; Kim et al., 2007; Lee et al., 2008; Kim et al., 2012). The use of biological control species is often ineffective when used alone in greenhouses infested with either multiple pests (in moderate densities) or a single pest species with a high density. Chemical control is a common option in such cases (Endo and Tsurumachi, 2001), thus making the application of chemical insecticides compatible with biological control species very important.

To this end, multiple chemical pesticides were evaluated for selectivity against biological control species used in pest control in South Korea. The selective toxicities of 42–83 pesticides had been assessed for *P. persimilis* (Ahn et al., 2004), *A. colemani* (Kim et al., 2006), and *Orius strigicollis*

Poppius (Choi et al., 2007). Many of the tested pesticides were toxic to these biological control species. Alternative biopesticides and environmentally friendly agricultural materials (EFAMs) have also been used in South Korea as an alternative to pesticides. However, many of these alternative pesticides were found to be toxic to biological control species. For example, many plant essential oils including caraway seed, citronella java, lemon eucalyptus, pennyroyal, peppermint, and spearmint oils were toxic to *P. persimilis*, causing > 90% mortality (Choi et al., 2004). The fumigant toxicity of 13 plant essential oils, i.e., pennyroyal, armoise, basil, cedarleaf, coriander, cypress, howood, hyssop, marjoram, myrtle, niaouli, rosemary, and sage oils, was high for *Thrips palmi* Karny, ranging from 2.63 to 19.21 mg/l (LC₅₀), but was low in *O. strigicollis*, varying from 17.29 to 158.22 mg/l (Yiet al., 2006). Of 61 EFAMs tested against *E. formosa*, *A. colemani*, *D. isaea*, and *Dacnusa sibirica* Telenga, many fungicidal EFAMs and EFAMs containing molybdenum were highly toxic, although insecticidal EFAMs were less so (Yu et al., 2006). The selective toxicities of many recently developed pesticides (bistrifluron, chlorfenapyr, chlorantraniliprole, cyantraniliprole, dinotefuran, emamectin benzoate, flubendiamide, indoxacarb, lepimecrtin, methoxyfenozide, pyridaben, spinetoram, and spinosad), which have been widely used to control pests in greenhouses,

have not been evaluated against pests' biological control species yet.

The objective of this study is to evaluate the contact and residual toxicity of those insecticides which have been widely used in greenhouse to control the pests instead of commercially biological control agents. A total of 13 insecticides were evaluated for the acute toxicity to seven species of biological control species (2 predatory mites, 3 hymenopteran parasitoids, and 2 hemipteran predators).

3.2. Materials and methods

3.2.1. Insects and mites

Two predatory mites (*P. persimilis* and *A. swirskii*), 3 hymenopteran parasitoids (*D. isaea*, *A. colemani*, and *E. formosa*), and 2 hemipteran predators (*O. laevigatus* and *N. tenuis*) were obtained from Dongbu Farm Ceres Co., Ltd in 2012, and were reared at 20-30°C in the greenhouse. Predatory mites were reared on kidney bean plants providing *T. urticae* as food. *D. isaea* was reared by feeding *Liriomyza trifolii* (Burgess) on cucumber plants. *A. colemani* and *E. formosa* were reared on *Aphis gossypii* Glover and *T. vaporariorum* on pepper plants, respectively. *O. laevigatus* and *N. tenuis* were reared on *T. vaporariorum* and the eggs of *Ephestia kuehniella* Zeller, respectively. For predators, the eggs that were laid on the same day were reared in the laboratory condition; young adults (< 5 days old) were collected directly from the colony. For parasitoids, collected pupae were stored in an incubator at 4 °C and before starting experiment, they were transferred to an incubator at 25 °C for adult emergence. Thus, young adults (< 5 days old) were used.

3.2.2. Contact toxicity

Insecticides, types of formulation, and their recommended concentrations used in this experiment were summarized in Table 3.1. For bioassay, 10-130 adult females (< 5 days old) of test species were placed in a Petri dish (100 mm diameter, 41 mm height) and sprayed with 1 ml of insecticide solution until exhaustion completely using a hand sprayer (5 ml cap.). All the experiments were replicated three times. Each insecticide solution was made by diluting insecticides with 100 ml of distilled water to make field-recommended concentrations (Table 3.1). Distilled water was used as a control. After spraying, the biological control species were dried for 10 minutes and kept at 25 ± 3 °C and a photoperiod of 16:8 (L:D) h in the laboratory. Mortality of individuals was checked after 48 h. Death was determined if the organism does not move > 1.5 times its body length when touched with a fine brush. Levels of toxicities of pesticides were judged by the IOBC standard classification: harmless (< 30%), slightly harmful (31–80%), moderately harmful (81–99%), and harmful (> 99%) (Karen and Croft, 1988; Choi et al., 2007).

3.2.3. Residual toxicity

Host plants for major pests which the respective biological control species feed on, i. e., strawberry for 2 predatory mites and paprika for 3

hymenopteran parasitoids and 2 hemipteran predators, were grown in the greenhouse for two months at 15-30°C. Host plants were sprayed with the recommended spray volume (1,500 l/ha) (Table 3.1) and dried naturally for 1 day. Leaves of host plants were collected at 1, 4, 7, 14, 21, and 28 days after treatment and used for the test. Ten grams of leaves were measured and placed in a Petri dish (100 mm diameter 41 mm height) and then 20-50 adult females per dish were placed with the food source and kept at 25 ± 3 °C on photoperiod of 16:8 (L:D) h in the laboratory for two days. Experiments were repeated three times for each pesticide. Death of individuals was checked after 48 h. Distilled water was used as a control. Residual toxicity of insecticides to biological control species was determined using the IOBC standard classification.

3.2.4. Data analysis

Corrected mortality percentage was calculated using Abbott's formula (Abbott, 1925):

$$\% \text{ corrected mortality} = \frac{X - Y}{X} \times 100$$

Where X = percentage survivorship in the control and Y = percentage survivorship in the treatment.

Percentage mortalities were arcsine-transformed before statistical analysis. A one-way analysis of variance (ANOVA) was conducted, followed by Tukey multiple-range tests at $P < 0.05$ for mean separation, using PROC GLM in SAS (SAS Institute, 2009).

Table 3.1. Lists of insecticides used in both contact and residual toxicity assay.

Common name	Target pests	Chemical group (IRAC MoA No.)	Formulation	AI(%)	Recommended conc.(ppm)
Bistrifluron	Lepidopterans, Whiteflies	Benzoylureas (15)	EC	10	50
Chlorfenapyr	Lepidopterans, Mites, Thrips	Chlorfenapyr (13)	SC	10	50
Chlorantraniliprole	Lepidopterans	Diamides (28)	WG	5	25
Cytrantraniliprole	Lepidopterans	Diamides (28)	OD	10	50
Dinotefuran	Sucking insects	Neonicotinoids (4A)	WP	10	100
Emamectin benzoate	Lepidopterans	Avermectins (6)	EC	2.15	10.75
Flubendiamide	Lepidopterans	Diamides (28)	WG	20	100
Indoxacarb	Lepidopterans	Indoxacarb (22A)	SC	5	50
Lepimectin	Mites, Leaf miners, Colorado beetles	Avermectins (6)	EC	2	10
Methoxyfenozide	Lepidopterans	Diacylhydrazines (18)	SC	21	105
Pyridaben	Whiteflies, Mites	METI acaricides (21A)	SC	20	133
Spinetoram	Lepidopterans, Thrips, Flies, Beetles, Grasshoppers	Spinosyns (5)	WG	5	25
Spinosad	Lepidopterans, Thrips, Flies, Beetles, Grasshoppers	Spinosyns (5)	SC	10	50

3.3. Results

3.3.1. Contact toxicity

Insecticides tested showed various levels of contact toxicity against biological control species (Table 3.2). Among the biological control species tested, predatory mites were relatively less susceptible to the direct spray of 6 insecticides (Table 3.2). Among the insecticides, dinotefuran, indoxacarb, chlorantraniliprole, cyantraniliprole, methoxyfenozide, and bistrifluron were harmless, and flubendiamide was slightly harmful to the predatory mites. But six insecticides (spinetoram, spinosad, lepimectin, chlorfenapyr, emamectin benzoate, and pyridaben) were moderately harmful showing >80% mortality within 48 h to *P. persimilis* ($F = 37.97$; $df = 12, 26$; $P < 0.001$) and to *A. swirskii* ($F = 265.10$; $df = 12, 26$; $P < 0.001$). In particular, indoxacarb, chlorantraniliprole, cyantraniliprole, flubendiamide, methoxyfenozide, and bistrifluron registered for the control of lepidopteran insects in South Korea (KCPA, 2018) were harmless to the predatory mites.

Three insecticides (chlorantraniliprole, methoxyfenozide, and bistrifluron) showed harmless to hemipteran predators, and cyantraniliprole was slightly harmful to *O. laevigatus* ($F = 55.15$; $df = 12, 26$; $P < 0.001$) and was harmless to *N. tenuis* ($F = 55.22$; $df = 12, 26$; $P < 0.001$) (Table 3.2).

However, five insecticides (dinotefuran, spinetoram, spinosad, emamectin benzoate, and pyridaben) were highly toxic and four insecticides (lepimectin, chlorfenapyr, indoxacarb, and flubendiamide) were slightly harmful to *O. laevigatus*. Three insecticides (dinotefuran, emamectin benzoate, and chlorfenapyr) were harmful, 2 insecticides (spinosad and pyridaben) were moderately harmful, and 4 insecticides (spinetoram, lepimectin, indoxacarb, and flubendiamide) were slightly harmful to *N. tenuis*.

All the insecticides tested were toxic to hymenopteran parasitoids except chlorantraniliprole and flubendiamide to *D. isaea* ($F = 144.35$; $df = 12, 26$; $P < 0.001$). Methoxyfenozide and bistrifluron were slightly harmful to *D. isaea*. Chlorantraniliprole and flubendiamide were only slightly harmful to *A. colemani* ($F = 46.27$; $df = 12, 26$; $P < 0.001$) and *E. formosa* ($F = 20.11$; $df = 12, 26$; $P < 0.001$), respectively.

Table 3.2. Mortality of 2 predatory mites, 2 hemipteran predators, and 3 hymenopteran parasitoids at 48 h after direct spray of various insecticides.

insecticides	Percent corrected mortality (Mean±SD)				
	<i>Phytoseiulus persimilis</i>	<i>Amblyseius swirskii</i>	<i>Orius laevigatus</i>	<i>Nesidiocoris tenuis</i>	
Bistrifluron	25.2 ± 29.2 b	4.0 ± 3.3 d	5.9 ± 4.3 e	0.0	d
Chlorfenapyr	100.0 a	98.4 ± 0.3 a	71.1 ± 9.8 bc	100.0	a
Chlorantraniliprole	9.3 ± 11.6 b	0.1 ± 0.2 c	4.5 ± 7.8 e	0.1 ± 0.2	d
Cyantraniliprole	0.0 c	3.8 ± 6.6 d	42.1 ± 12.2 d	0.0	d
Dinotefuran	21.7 ± 3.0 b	18.9 ± 8.3 c	100.0 a	100.0	a
Emamectin benzoate	100.0 a	95.9 ± 1.9 a	100.0 a	100.0	a
Flubendiamide	37.2 ± 15.7 b	20.1 ± 6.0 c	42.1 ± 11.7 d	54.3 ± 13.1	c
Indoxacarb	9.1 ± 12.9 b	3.0 ± 5.3 d	41.9 ± 15.7 d	78.2 ± 14.6	abc
Lepimectin	100.0 a	92.3 ± 6.7 b	61.3 ± 13.6 cd	65.9 ± 21.1	bc
Methoxyfenozide	25.9 ± 21.6 b	8.4 ± 0.6 cd	6.4 ± 8.6 e	12.3 ± 12.9	d
Pyridaben	100.0 a	82.7 ± 4.9 b	100.0 a	90.2 ± 9.1	ab
Spinetoram	97.0 ± 5.2 a	90.7 ± 4.0 b	93.2 ± 7.8 b	79.9 ± 7.4	abc
Spinosad	99.3 ± 1.2 a	95.9 ± 3.5 b	100.0 a	81.9 ± 6.7	abc

Table 3.2. Continued.

insecticides	Percent corrected mortality (Mean±SD)			
	<i>Diglyphus isaea</i>	<i>Aphidius colemani</i>	<i>Encarsia formosa</i>	
Bistrifluron	48.5 ± 9.4	b	100.0	a
Chlorfenapyr	100.0	a	100.0	a
Chlorantraniliprole	7.6 ± 5.8	c	36.8 ± 13.5	b
Cyantraniliprole	85.3 ± 14.3	a	100.0	a
Dinotefuran	100.0	a	100.0	a
Emamectin benzoate	100.0	a	100.0	a
Flubendiamide	0.0	c	90.3 ± 8.0	a
Indoxacarb	100.0	a	100.0	a
Lepimectin	100.0	a	100.0	a
Methoxyfenozide	58.1 ± 1.1	b	96.4 ± 3.4	a
Pyridaben	94.5 ± 5.7	a	100.0	a
Spinetoram	100.0	a	100.0	a
Spinosad	100.0	a	100.0	a

3.3.2. Residual toxicity

Insecticides showed different residual toxicity depending on biological control species (Table 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, and 3.9). All of the insecticides tested except chlorfenapyr and spinetoram were harmless at 1st day ($F = 26.76$; $df = 12, 26$; $P < 0.001$; Table 3.3), and spinetoram became harmless at 4th days to *P. persimilis*. However, the residual toxicity of chlorfenapyr to *P. persimilis* was very high and persisted until 7 days after the application. Eleven insecticides except spinosad and lepimectin were harmless at 1st day ($F = 26.47$; $df = 12, 26$; $P < 0.001$; Table 3.4) and spinosad and lepimectin became harmless to *A. swirskii* at 4th days.

Seven insecticides (lepimectin, chlorfenapyr, chlorantraniliprole, cyantraniliprole, flubendiamide, methoxyfenozide, bistrifluron, and pyridaben) were harmless at 1st day ($F = 64.80$; $df = 12, 26$; $P < 0.001$; Table 3.5), and emamectin benzoate and indoxacarb became harmless to *O. laevigatus* at 4th days after application ($F = 183.79$; $df = 12, 26$; $P < 0.001$). However, four insecticides (dinotefuran, spinetoram, spinosad, and pyridaben) showed high toxicity. In particular, the toxicity of dinotefuran to *O. laevigatus* persisted until 28 days. Most insecticides except dinotefuran, chlorfenapyr, and indoxacarb were harmless at 4th day to *N. tenuis* ($F = 41.72$; $df = 12, 26$; $P < 0.001$; Table 3.6). Among the insecticides tested, dinotefuran has

the highest toxicity to hemipteran predators.

Six insecticides (Indoxacarb, chlorantraniliprole, cyantraniliprole, flubendiamide, methoxyfenozide, and bistrifluron) were harmless to *D. isaea* at 1st day ($F = 31.70$; $df = 12, 26$; $P < 0.001$; Table 3.7), and the residual toxicities of the other insecticides persisted until 7 days ($F = 2.34$; $df = 12, 26$; $P < 0.001$). Two insecticides (methoxyfenozide and bistrifluron) were harmless to *A. colemani* at 1st day ($F = 10.02$; $df = 12, 26$; $P < 0.001$; Table 3.8) and 4 insecticides (emamectin benzoate, chlorantraniliprole, cyantraniliprole, and flubendiamide) were harmless at 4th days ($F = 118.20$, $df = 12, 26$, $P < 0.001$). Only cyantraniliprole was harmless to *E. formosa* at 1st day ($F = 11.40$; $df = 12, 26$; $P < 0.001$; Table 3.9) and flubendiamide and bistrifluron became harmless at 4th day ($F = 29.08$; $df = 12, 26$; $P < 0.001$).

Table 3.3. Mortality of *P. persimilis* at 1, 4, and 7 days after application.

Pesticides	Percent corrected mortality (Mean±SD)		
	1DAT	4DAT	7DAT
Bistrifluron	21.1 ± 7.9	bc	0.0
Chlorfenapyr	98.2 ± 3.1	a	100.0
Chlorantraniliprole	21.7 ± 8.1	bc	0.0
Cyantraniliprole	3.4 ± 3.0	c	0.0
Dinotefuran	21.2 ± 13.5	bc	0.0
Emamectin benzoate	27.3 ± 14.4	bc	0.0
Flubendiamide	0.0 ± 0.0	c	0.0
Indoxacarb	12.9 ± 3.5	bc	0.0
Lepimectin	15.0 ± 4.4	bc	0.0
Methoxyfenozide	12.3 ± 5.5	bc	0.0
Pyridaben	15.7 ± 20.9	bc	0.0
Spinetoram	34.4 ± 6.0	b	15.1 ± 9.4
Spinosad	25.3 ± 5.5	bc	15.2 ± 11.1

Table 3.4. Mortality of *A. swirskii* at 1 and 4 days after application.

Pesticides	Percent corrected mortality (Mean±SD)	
	1DAT	4DAT
Bistrifluron	0.1 ± 0.2 c	0.0 b
Chlorfenapyr	8.2 ± 7.5 c	0.0 b
Chlorantraniliprole	1.6 ± 1.8 c	0.0 b
Cyantraniliprole	9.5 ± 6.6 c	0.0 b
Dinotefuran	1.2 ± 1.1 c	0.0 b
Emamectin benzoate	6.4 ± 2.0 c	0.0 b
Flubendiamide	6.3 ± 5.2 c	0.0 b
Indoxacarb	7.0 ± 7.2 c	0.0 b
Lepimectin	33.9 ± 12.1 b	10.8 ± 2.4 a
Methoxyfenozide	1.4 ± 1.6 c	0.0 b
Pyridaben	7.1 ± 3.2 c	0.0 b
Spinetoram	0.8 ± 0.7 c	0.0 b
Spinosad	54.4 ± 4.3 a	6.3 ± 9.5 a

Table 3.5. Mortality of *O. laevigatus* at 1, 4, 7, 14, 21, and 28 days after application.

Pesticides	Percent corrected mortality (Mean±SD)		
	1DAT	4DAT	7DAT
Bistrifluron	23.0 ± 8.4 c	0.0	d 0.0 b
Chlorfenapyr	17.5 ± 27.1 c	0.0	d 0.0 b
Chlorantraniliprole	3.0 ± 5.0 c	0.0	d 0.0 b
Cyantraniliprole	11.9 ± 6.4 c	0.0	d 0.0 b
Dinotefuran	100.0	100.0	a 98.1 ± 3.3 a
Emamectin benzoate	63.0 ± 12.3 b	4.0 ± 3.4	c 0.0 b
Flubendiamide	0.0 ± 0.0 c	0.0	d 0.0 b
Indoxacarb	77.9 ± 4.5 ab	1.5 ± 2.7	c 0.0 b
Lepimectin	4.9 ± 8.3 c	0.0	d 0.0 b
Methoxyfenozide	4.8 ± 3.3 c	0.0	d 0.0 b
Pyridaben	75.5 ± 10.2 ab	74.5 ± 6.8	b 90.3 ± 6.6 a
Spinetoram	100.0	90.6 ± 11.7 ab	a 85.6 ± 8.0 a
Spinosad	100.0	98.1 ± 3.2	a 96.4 ± 3.1 a

Table 3.5. *Continued*

Pesticides	Percent corrected mortality (Mean±SD)			
	14DAT	21DAT	28DAT	
Bistrifluron	0.0	0.0	c	0.0
Chlorfenapyr	0.0	0.0	c	0.0
Chlorantraniliprole	0.0	0.0	c	0.0
Cyantraniliprole	0.0	0.0	c	0.0
Dinotefuran	88.8 ± 5.4	84.5 ± 13.7	a	90.8 ± 8.3
Emamectin benzoate	0.0	0.0	c	0.0
Flubendiamide	0.0	0.0	c	0.0
Indoxacarb	0.0	0.0	c	0.0
Lepimectin	0.0	0.0	c	0.0
Methoxyfenozide	0.0	0.0	c	0.0
Pyridaben	10.7 ± 13.9	0.0	c	0.0
Spinetoram	68.8 ± 3.4	12.1 ± 5.2	ab	0.0
Spinosad	38.8 ± 24.2	17.2 ± 5.2	c	0.0

Table 3.6. Mortality of *N. tenuis* at 1, 4, 7, 14, and 21 days after application.

Pesticides	Percent corrected mortality (Mean±SD)				
	1DAT	4DAT	7DAT		
Bistrifluron	11.2 ± 7.7 def	0.0	d	0.0	c
Chlorfenapyr	96.8 ± 2.8 a	70.8 ± 8.9	a	9.0 ± 5.0	b
Chlorantraniliprole	7.0 ± 1.8 ef	0.0	d	0.0	c
Cyantraniliprole	12.8 ± 7.6 def	0.0	d	0.0	c
Dinotefuran	100.0	69.9 ± 30.6	a	93.9 ± 6.5	a
Emamectin benzoate	23.4 ± 2.9 cdef	0.0	d	0.0	c
Flubendiamide	45.4 ± 22.6 bc	25.5 ± 16.0	bc	0.0	c
Indoxacarb	66.3 ± 12.2 b	66.8 ± 5.5	ab	7.5 ± 2.8	b
Lepimectin	8.5 ± 4.2 ef	0.0	d	0.0	c
Methoxyfenozide	4.4 ± 2.2 f	0.0	d	0.0	c
Pyridaben	32.0 ± 1.9 cde	0.0	d	0.0	c
Spinetoram	36.3 ± 4.5 cd	9.2 ± 8.3	c	0.0	c
Spinosad	27.4 ± 12.5 cdef	13.7 ± 5.8	c	0.0	c

Table 3.6. *Continued*

Pesticides	Percent corrected mortality (Mean±SD)	
	14DAT	21DAT
Bistrifluron	0.0	b 0.0 b
Chlorfenapyr	0.0	b 0.0 b
Chlorantraniliprole	0.0	b 0.0 b
Cyantraniliprole	0.0	b 0.0 b
Dinotefuran	79.7 ± 22.9	a 3.6 ± 3.5 a
Emamectin benzoate	0.0	b 0.0 b
Flubendiamide	0.0	b 0.0 b
Indoxacarb	0.0	b 0.0 b
Lepimectin	0.0	b 0.0 b
Methoxyfenozide	0.0	b 0.0 b
Pyridaben	0.0	b 0.0 b
Spinetoram	0.0	b 0.0 b
Spinosad	0.0	b 0.0 b

Table 3.7. Mortality of *D. isaea* at 1, 4, 7, 14, 21, and 28 days after application.

Pesticides	Percent corrected mortality (Mean±SD)				
	1DAT	4DAT	7DAT		
Bistrifluron	5.4 ± 5.3	0.0	0.0	c	b
Chlorfenapyr	98.5 ± 2.6	98.2 ± 3.1	93.3 ± 7.3	a	a
Chlorantraniliprole	12.9 ± 12.5	0.0	0.0	c	b
Cyantraniliprole	8.2 ± 10.5	0.0	0.0	c	b
Dinotefuran	78.8 ± 20.1	56.3 ± 13.2	65.3 ± 13.8	ab	a
Emamectin benzoate	34.7 ± 5.4	29.5 ± 6.2	48.0 ± 25.4	b	a
Flubendiamide	7.6 ± 10.4	0.0	0.0	c	b
Indoxacarb	8.8 ± 3.3	0.0	0.0	c	b
Lepimectin	79.9 ± 14.7	64.3 ± 43.0	88.4 ± 10.1	ab	a
Methoxyfenozide	4.9 ± 4.8	0.0	0.0	c	b
Pyridaben	69.0 ± 28.9	57.2 ± 7.2	48.5 ± 23.4	ab	a
Spinetoram	95.2 ± 4.6	84.1 ± 21.1	59.6 ± 37.7	a	a
Spinosad	96.7 ± 5.8	100.0	76.7 ± 1.9	a	a

Table 3.7. *Continued*

Pesticides	Percent corrected mortality (Mean±SD)				
	14DAT	21DAT	28DAT		
Bistrifluron	0.0	c	0.0	c	0.0
Chlorfenapyr	79.8 ± 12.3	a	50.2 ± 9.09	a	11.8 ± 20.4
Chlorantraniliprole	0.0	c	0.0	c	0.0
Cyantraniliprole	0.0	c	0.0	c	0.0
Dinotefuran	11.8 ± 6.2	b	0.0	c	0.0
Emamectin benzoate	1.4 ± 2.8	b	0.0	c	0.0
Flubendiamide	0.0	c	0.0	c	0.0
Indoxacarb	0.0	c	0.0	c	0.0
Lepimectin	16.5 ± 12.1	b	0.0	c	0.0
Methoxyfenozide	0.0	c	0.0	c	0.0
Pyridaben	9.9 ± 6.2	b	0.0	c	0.0
Spinetoram	61.0 ± 26.1	a	6.0 ± 6.10	b	0.0
Spinosad	84.0 ± 15.1	a	0.6 ± 0.98	b	0.0

Table 3.8. Mortality of *A. colemani* at 1, 4, 7, 14, 21, and 28 days after application.

Pesticides	Percent corrected mortality (Mean±SD)		
	1DAT	4DAT	7DAT
Bistrifluron	15.6 ± 33.2 b	0.0	d 0.0
Chlorfenapyr	100.0	100.0	a 100.0
Chlorantraniliprole	39.5 ± 17.5 b	3.1 ± 8.0 c	d 0.0
Cyantraniliprole	59.3 ± 20.1 ab	19.0 ± 13.5 bc	d 0.0
Dinotefuran	100.0	100.0	a 100.0
Emamectin benzoate	97.4 ± 4.5 a	24.1 ± 6.2 b	d 0.0
Flubendiamide	53.8 ± 41.1 ab	24.5 ± 8.8 b	d 0.0
Indoxacarb	94.1 ± 5.8 a	87.4 ± 5.8 a	83.1 ± 3.2 c
Lepimectin	98.4 ± 2.7 a	92.8 ± 9.9 a	93.6 ± 2.3 b
Methoxyfenozide	22.3 ± 25.9 b	0.0	d 0.0
Pyridaben	100.0	100.0	a 100.0
Spinetoram	100.0	100.0	a 100.0
Spinosad	100.0	100.0	a 100.0

Table 3.8. *Continued*

Pesticides	Percent corrected mortality (Mean±SD)				
	14DAT		21DAT		28DAT
Bistrifluron	0.0	c	0.0	d	0.0
Chlorfenapyr	100.0	a	100.0	a	100.0
Chlorantraniliprole	0.0	c	0.0	d	0.0
Cyantraniliprole	0.0	c	0.0	d	0.0
Dinotefuran	100.0	a	100.0	a	100.0
Emamectin benzoate	0.0	c	0.0	d	0.0
Flubendiamide	0.0	c	0.0	d	0.0
Indoxacarb	73.2 ± 11.0	b	61.2 ± 15.2	b	57.3 ± 11.0
Lepimectin	89.1 ± 11.1	ab	15.4 ± 5.2	c	0.0
Methoxyfenozide	0.0	c	0.0	d	0.0
Pyridaben	100.0	a	100.0	a	97.8 ± 2.0
Spinetoram	100.0	a	100.0	a	100.0
Spinosad	100.0	a	97.8 ± 3.8	a	94.0 ± 5.6

Table 3.9. Mortality of *E. formosa* at 1, 4, 7, 14, 21, and 28 days after application.

Pesticides	Percent corrected mortality (Mean±SD)					
	1DAT	4DAT			7DAT	
Bistrifluron	43.3 ± 22.1	bc	20.2 ± 2.8	ef	0.0	f
Chlorfenapyr	100.0	a	100.0	a	73.0 ± 6.6	bc
Chlorantraniliprole	71.9 ± 9.6	ab	52.6 ± 9.4	cd	4.0 ± 2.5	e
Cyantraniliprole	24.3 ± 13.0	c	0.0	g	0.0	f
Dinotefuran	91.3 ± 3.9	a	83.5 ± 7.5	ab	56.7 ± 10.3	cd
Emamectin benzoate	98.0 ± 3.4	a	79.5 ± 12.0	abc	19.2 ± 8.4	e
Flubendiamide	82.1 ± 31.0	a	13.6 ± 6.5	f	0.0	f
Indoxacarb	88.5 ± 5.1	a	61.5 ± 11.9	bcd	4.6 ± 6.1	e
Lepimectin	100.0	a	100.0	a	94.1 ± 7.5	ab
Methoxyfenozide	81.9 ± 6.0	a	49.3 ± 4.9	de	2.4 ± 2.5	e
Pyridaben	100.0	a	100.0	a	100.0	a
Spinetoram	72.5 ± 10.4	ab	37.2 ± 25.1	def	46.2 ± 13.5	d
Spinosad	100.0	a	90.9 ± 8.8	ab	81.6 ± 8.6	ab

Table 3.9. *Continued*

Pesticides	Percent corrected mortality (Mean±SD)		
	14DAT	21DAT	28DAT
Bistrifluron	0.0	d 0.0	d 0.0
Chlorfenapyr	53.8 ± 12.8	bc 6.4 ± 3.0	c 0.0
Chlorantraniliprole	0.0	d 0.0	d 0.0
Cyantraniliprole	0.0	d 0.0	d 0.0
Dinotefuran	41.3 ± 9.3	c 10.3 ± 6.2	c 0.0
Emamectin benzoate	0.0	d 0.0	d 0.0
Flubendiamide	0.0	d 0.0	d 0.0
Indoxacarb	0.0	d 0.0	d 0.0
Lepimectin	72.9 ± 12.5	ab 8.8 ± 4.2	c 0.0
Methoxyfenozide	0.0	d 0.0	d 0.0
Pyridaben	89.3 ± 7.2	a 73.3 ± 7.7	a 43.8 ± 7.0
Spinetoram	53.9 ± 5.2	bc 58.5 ± 8.2	ab 48.5 ± 8.3
Spinosad	62.1 ± 3.3	bc 44.6 ± 10.6	b 51.0 ± 5.3

3.4. Discussion

The toxicities to adult females than other stages should provide useful information to IPM programs because biological control species released as an adult form commercially to control greenhouse pests. In this study, we found that the toxicities (contact and residual) of insecticides against biological control species of common greenhouse pests differed significantly among control species and among insecticides. In general, predatory mites and hemipteran predators showed less susceptibility to pesticides than hymenopteran parasitoids. For hemipteran predators, dinotefuran which is commonly used in greenhouses to manage sucking insects such as aphids and whiteflies was known to have higher contact toxicity and longer residual toxicity than spinosyn insecticides (Cloyd and Bethke, 2011). In addition, imidacloprid which belong to the same chemical group of dinotefuran tested in this study was reported to be toxic to various hymenopteran parasitoids (Bacci et al., 2007; Preetha et al., 2009) and results generally agreed with these reports. In our study, neonicotinoid and spinosyn insecticides also showed high residual toxicity to *E. formosa* and *O. laevigatus*. Sterk et al. (2003) also reported that neonicotinoids (imidacloprid and thiamethoxam) were very toxic to *E. Formosa*. However, neonicotinoid insecticides were harmless to predatory mites in both contact and residual toxicity tests; thus,

they can be used in combination with predatory mites as a biological control species (Margaret et al., 2010; Huang et al., 2015).

Acetinomycete products such as spinosad and spinetoram are known to be very harmful to most small insects (All and Treacy, 2006). In our study, these spinosyn insecticides showed high contact toxicity to all the biological control species tested with relatively high and long residual toxicity to *E. formosa* and *O. laevigatus*. Tillman and Mulrooney (2000) reported very similar finding that spinosad showed high topical toxicity to hymenopteran parasitoids and hemipteran predators and high residual toxicity to hymenopteran parasitoids. Miles (2006) also showed that spinosad has high residual toxicity, persisting for at least 14 days in greenhouses. Therefore, these spinosyn insecticides can be classified as non-selective insecticides against hymenopteran parasitoids.

A previous study (Haseeb and Amano, 2002) showed that chlorfenapyr and emamectin benzoate, which belong to the same chemical group with lepimectin, were highly toxic to *Cotesia plutellae* Kurd. In our study, contact and residual toxicities of chlorfenapyr were very high and residual toxicity persisted until 7 days after application to *P. persimilis*. However, Kim and Yoo (2002) reported that chlorfenapyr was less toxic to adult female (98% survivorship at 24 h) and immature (70% survivorship) *P.*

persimilis. We speculate that potential genetic variation of *P. persimilis* populations might be responsible for this discrepancy. Lepimectin, a fermentation product of soil microorganisms, showed high contact toxicity to all of the biological control species tested in our study. However, lepimectin was harmless to *P. persimilis* and *O. laevigatus* in the residual toxicity test. Abamectin, which belongs to the same chemical group with lepimectin, also showed the similar pattern and did not significantly affect the survival or mobility of *P. persimilis* in the residual toxicity assay (Zhang and Sanderson, 1990).

Chlorantraniliprole, cyantraniliprole, flubendiamide, indoxacarb, methoxyfenozide, and bistrifluron which were registered for the control of lepidopteran insects (KCPA, 2018) showed low contact and low residual toxicities against predatory mites and hemipteran predators, and thus can be classified as selective insecticides for biological control species. In a previous study (Angeli et al., 2005), diacylhydrazine (methoxyfenozide) was shown to be less toxic than imidacloprid. Bostanian and Akalach (2006) presented similar results to our study that indoxacarb had no toxicity to *Orius insidiosus* Say nymphs, *Amblyseius fallacies* Garman adults, and *P. persimilis* adults. Similarly, Roubos et al. (2014) showed that the selective insecticides including chlorantraniliprole, methoxyfenozide, and indoxacarb

had low residual toxicity against *A. colemani*, *O. insidiosus*, *Hippodamia convergens* Guérin-Méneville, and *Chrysoperla rufilabris* Burmeister 3 days after treatment. In addition, chlorantraniliprole was safe in contact toxicity to *D. isaea* (a hymenopteran parasitoid), and showed a relatively short residual toxicity to *E. formosa*. The result in our study was similar to the study that reported chlorantraniliprole was harmless to *Trichogramma chilonis* Ishii (Preetha et al., 2009). Thus, chlorantraniliprole may also be used for some parasitoid species.

This study provides information of selective insecticides based on the contact and residual toxicities against biological control species. Evaluating the acute toxicities in laboratory condition may not accurately indicate how they would perform in field condition. However, the results may be used for future studies and provide basic information for biological control based IPM.

Chapter 4.

Comparison of three application methods of chlorantraniliprole in pepper

Abstract

To find out the effective application method for a diamide insecticide, chlorantraniliprole, which has the longest residual toxicity against lepidopteran insect in Chapter 2 and systemic properties, toxicities of three application methods (the foliar spray, the foliar spray mixed with a wetting agent, and the soil drenching) were investigated in the greenhouse on red pepper, *Capsicum annuum*, and comparison was made for the residue levels of chlorantraniliprole on leaves and fruits,. The highest toxicity was found in the foliar spray and the foliar spray mixed with a wetting agent. In the foliar spray, residue of chlorantraniliprole was highest at the same day treated and then began to decline quickly compared to other application methods. However, the toxicity and the residue in foliar spray mixed with a wetting agent remained stable. The residue in soil drenching increased until 28 days and then declined. The decline patterns of chlorantraniliprole residue were similar between fruits and leaves, but the residue was lower from 34 to 61-fold in fruits than in leaves. To control lepidopteran insect pests, foliar spray was a best application method. However, diverse application methods could be used to take advantage of the systemic characteristic of chlorantraniliprole, if useful and proper information should be given to farmers.

Keywords: application method, foliar spray, foliar spray mixed with a wetting agent, soil drenching, chlorantraniliprole

4.1. Introduction

Control methods against lepidopteran insect pests in ornamental and greenhouse crops have been studied for increasing the efficacy of insecticides and for reducing insecticide resistance due to its repeated applications (Kim et al., 2003; Saleem et al., 2016). Farmers are using various application methods to reduce input costs and harvest more yields (Byrne et al., 2010; Cameron et al., 2015). The IPM system was introduced to reduce overuse of insecticides and development of insecticide resistance (Rehan and Freed, 2014; Muriithi et al., 2016). However, insecticide has been used as the primary strategy to control the lepidopteran insect pests. Repeated spray and overuse of insecticides caused development of insecticide resistance to numerous conventional insecticides and disrupted control strategies (Ahmad et al., 2007; Saleem et al., 2016). To mitigate the insecticide resistance problem, various application methods such as rotation of insecticides with different mode of action, and combined use of synergists such as piperonyl butoxide (PBO), diethyl-maleate (DEM), and triphenyl phosphate (TPP) have been recommended (Muthusamy et al., 2014).

Chlorantraniliprole, an anthranilic diamide insecticide, is one of the most important insecticides for the control of lepidopteran insects in South

Korea (KCPA, 2018). Chlorantraniliprole binds to ryanodine receptors (RyR), leading to uncontrolled calcium release in insect muscles and exhibiting low toxicity against non-target organisms such as birds, fishes, mammals, and soil invertebrates (Lahm et al., 2009, Lavtižar et al., 2016). Systemic characteristics of chlorantraniliprole is advantageous in root uptake application methods (Juraske et al., 2009; Cameron et al., 2015). Although there were recent reports of resistance development to *Spodoptera litura*, *Spodoptera exigua*, and *Plutella xylostella* (Ahmad et al., 2007; Tiancai et al., 2011; Zhen-di et al., 2014; Saleem et al., 2016), Chlorantraniliprole is still the most effective insecticide for controlling lepidopteran pests. Chlorantraniliprole is formulated and labeled for the use in foliar and soil application in South Korea (KCPA, 2018) and has been used in a variety of soil application methods in other countries (Cameron et al., 2015). Systemic application is an effective method for controlling lepidopteran insect pests in greenhouse cultivation systems. It reduces application time by lengthening persistence of active ingredient in plants. Foliar application can cause up to five times higher residue than soil irrigation (Juraske et al., 2009).

The objective of this study was to find effective application methods of chlorantraniliprole which has been highly toxic to *S. litura*, but harmless to biological control species as shown in Chapter 3. Chlorantraniliprole was

treated by three application methods, i.e., foliar spray, foliar spray mixed with a wetting agent, and soil drenching, and toxicities and residues were evaluated against *S. litura* in the laboratory condition.

4.2. Materials and methods

4.2.1. Host plant and insect colony

Red pepper, *Capsicum annuum*, was grown in the greenhouse for one month at 15-30°C. *Spodoptera litura*, originally obtained from Chungbuk National University in 2005, was reared with artificial diets (Goh et al., 1990) under standard laboratory condition of 25±1°C, a photoperiod of 16:8 (L:D) h, and 65% RH. During rearing in the laboratory, *S. litura* was not exposed to any insecticides. The 2nd instar larvae were collected and used for the bioassays.

4.2.2. Toxicity assay

Chlorantraniliprole manufactured as 5% wettable granule formulation was selected as a test chemical among the insecticides tested in Chapter 3. The formulated chlorantraniliprole was diluted in distilled water to make field recommended concentration (50 g active ingredient/ha). Three application methods, foliar spray, foliar spray mixed with wetting agent (spray volume 2,000 L/ha), and soil drenching, were chosen and treated on red pepper (Table 4.1). Distilled water was used as a control. Leaves of red pepper treated were collected at 0, 3, 7, 21, 28, and 42 days after application and

were punched to make leaf disc (50 mm diameter). For toxicity assay, ten 2nd instar stages of *S. litura* were placed on the leaf discs and placed on the insect breeding dish (100 × 41 mm dimension). Death was determined if larvae does not move > 1.5 times its body length when touched with a fine brush. All the experiments were replicated by three times and conducted in standard laboratory condition as described above.

4.2.3. Residue analysis of chlorantraniliprole

For the residual analysis, leaves and fruits of red pepper (10 g per sample) treated with chlorantraniliprole were grinded and then transferred into polypropylene centrifuge tube with acetonitrile (100 mL) (HPLC grade, Burdick & Jackson, USA). The tubes were capped and shaken on a mechanical shaker (KMC-1205S, Vision Scientific Co, KOR) for 30 min. The samples were allowed to be filtered through celite 545 and remnants were rinsed with acetonitrile (50 ml) and then were evaporated to dryness by using rotary vacuum evaporator (B-481, Büchi Co., CHE). After moving a separatory funnel, the samples were saturated with NaCl solution (50 ml) and distilled water (50 ml), distributed with dichloromethane to two volumes (100 and 50 ml), and then removed water passing through sodium sulfate (Na₂SO₄) anhydrous (Reagent grade, Junsei Chemical Co., JPN). The

extracts were evaporated to dry condition and then dissolved in *n*-hexane/acetone (90/10, v/v) (10 ml). The samples were loaded in chromatography column (70 cm × 1.4 cm i.d.) which was packed with Florisil (10 g) of activated *n*-hexane (HPLC grade, Burdick & Jackson, USA). Loaded column was washed by *n*-hexane/acetone (90/10, v/v) (100 ml) and eluted by *n*-hexane/acetone (70/30, v/v) (100 ml). The samples eluted were concentrated by using rotary vacuum evaporator, re-solved in *n*-hexane/acetone (90/10, v/v) (5 ml), and then activated by *n*-hexane (10 ml). They were loaded in Florisil SPE cartridge (1 g, 6 cc, Agilent, USA). The loaded samples were swilled by *n*-hexane/acetone (90/10, v/v) (10 ml) and eluted *n*-hexane/ethyl acetate (70/30, v/v) (20 ml). The samples were concentrated by rotary vacuum evaporator, adjusted to 2 ml final volume with acetonitrile (2 ml), and inserted to UPLC/MSD (Xevo TQ, Waters, USA). Recovery rates of chlorantraniliprole of each 10 g in 0.2 ppm and 1.0 ppm on fruits and leaves were 84.2 – 92.7% (Table 4.2) and 82.0 – 87.2% (Table 4.3), respectively. Precursor ion and product ion of chlorantraniliprole in UPLC/MSD were 484.054 *m/z* (cone voltage 18 V) and 111.954 and 453.152 *m/z* (collision voltage 60 V, 18 V), respectively. The retention times were at 1.4 min in fruits (Fig. 4.1) and 6.1 min in leaves (Fig. 4.2). All the samples of fruits and leaves were checked for the peaks which were

measured in the area in chromatogram to monitor chlorantraniliprole levels at 0, 3, 7, 21, 28, and 42 days after application. All the experiments were replicated by three times.

Table 4.1. Lists of insecticide and application methods in toxicity and residual analysis.

Common name	Formulation	AI (%)	Recommended conc. (g a.i./ha)	Application methods
Chlorantraniliprole	WG	5	50	Foliar spray
				Foliar spray mixed with a wetting agent (siloxane 30% SL)
				Soil drenching

Table 4.2. Recovery rate (%) of chlorantraniliprole in fruits of red pepper.

Common name	Dose (ppm)	2 hours after application			
		1	2	3	Mean \pm SD
Chlorantraniliprole	0.2	86.4	92.7	85.7	88.3 \pm 3.9
	1.0	84.2	85.1	84.9	84.7 \pm 0.5

Table 4.3. Recovery rate (%) of chlorantraniliprole in leaves of red pepper.

Common name	Dose (ppm)	2 hours after application			
		1	2	3	Mean \pm SD
Chlorantraniliprole	0.2	85.3	82.4	87.2	85.0 \pm 2.4
	1.0	82.0	84.1	83.2	83.1 \pm 1.1

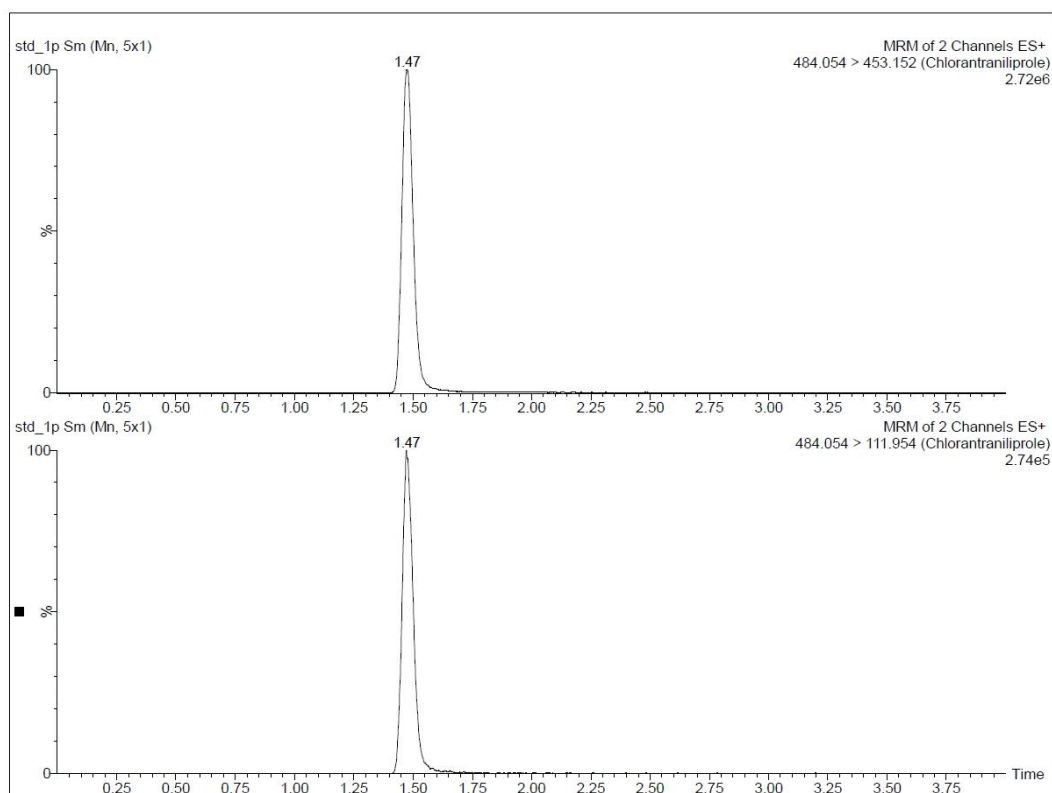


Fig. 4.1. Chromatogram of chlorantraniliprole in fruits (standard: 1.0 ppm).

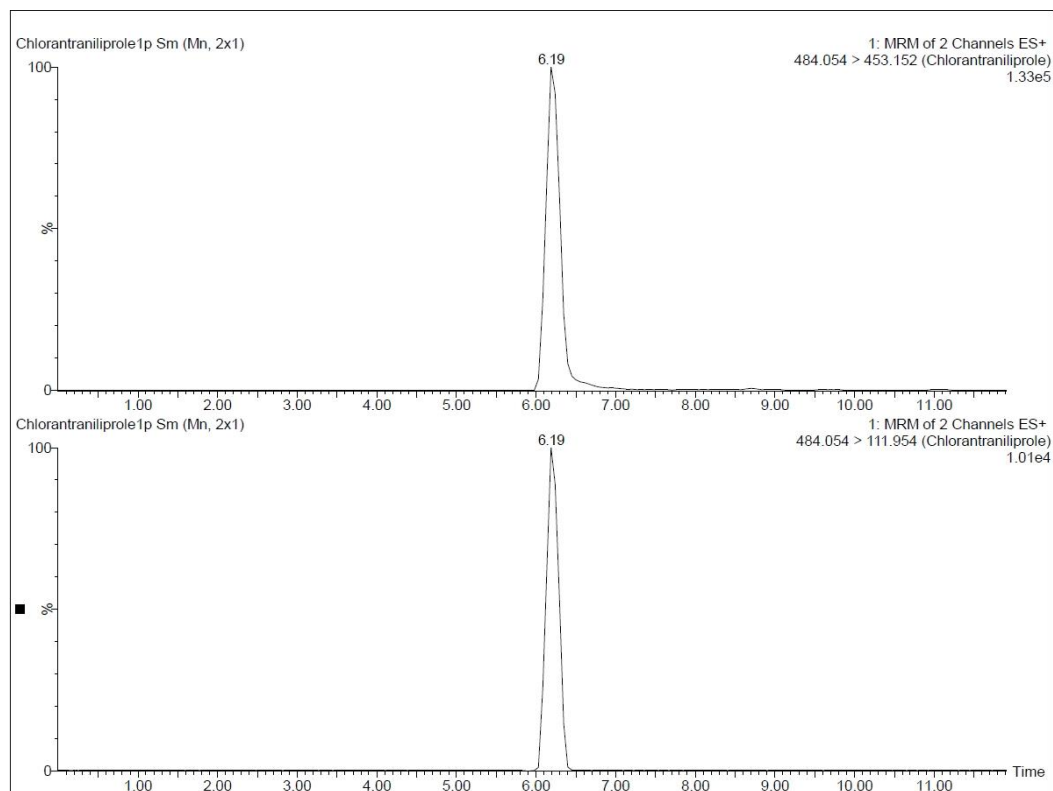


Fig. 4.2. Chromatogram of chlorantraniliprole in leaves (standard: 1.0 ppm).

4.3. Results

The toxicities and the mean residues of chlorantraniliprole on leaf tissue samples were measured (Fig. 4.3). Residues of chlorantraniliprole in each sample ($n = 3$, 10 g per sampling date) were analyzed by UPLC/MSD. The highest toxicity was shown in the foliar spray until 28 days after treatment, causing 100% mortality, followed by the foliar spray mixed with a wetting agent which remained high toxicity until 21 days. Chlorantraniliprole residues in the foliar spray and the foliar spray mixed with a wetting agent peaked at 7.04 ppm and 2.77 ppm at the same time of chlorantraniliprole treated and then gradually decreased to 1.12 ppm and 1.59 ppm, respectively. But the residue remained lower in the foliar spray mixed with a wetting agent than in the foliar spray. The toxicity in soil drenching was shown from 3 days after application and then remained stable until 28 days and the concentration of residue was also shown the similar pattern with the toxicity assay.

The residue patterns of chlorantraniliprole were similar between fruits and leaves in the foliar spray and the foliar spray mixed with a wetting agent (Fig. 4. 4a and b). The concentration on leaves was higher than fruits. In the foliar spray, residue of chlorantraniliprole on fruits was lower in 34 and 61-fold

than residue in leaves at 0 and 21 days, respectively (Fig. 4.4a). Residue on fruits in the foliar spray mixed with a wetting agent remained more stable than in the foliar spray (Fig. 4.4b).

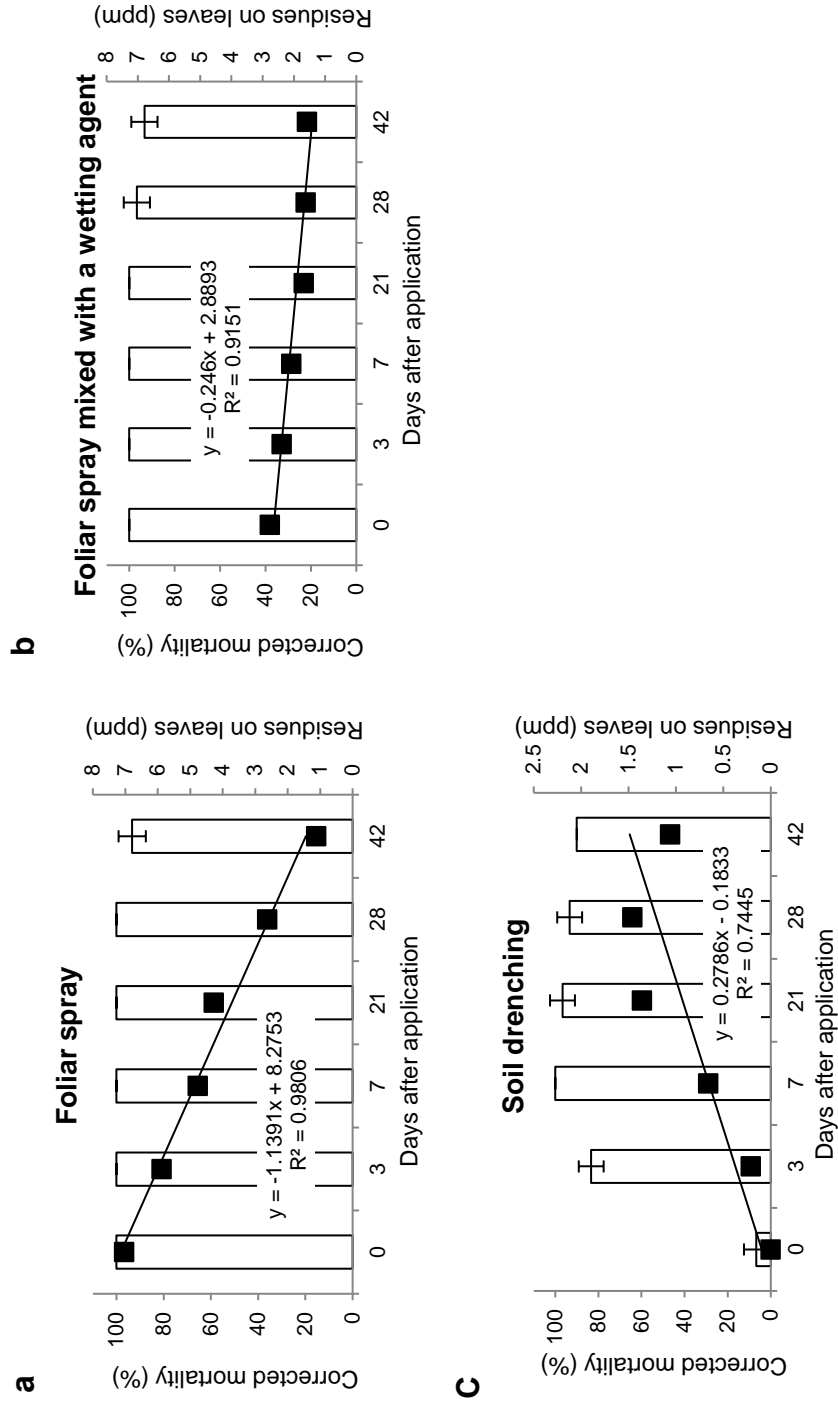


Fig. 4.3. Corrected mortalities of *S. litura* 2nd instar stages and residues in leaves treated with foliar spray, foliar spray mixed with a wetting agent and soil drenching of chlorantraniliprole. Each bar represents the mortality (\pm SEM). Each point represents the residue concentration (ppm) of chlorantraniliprole.

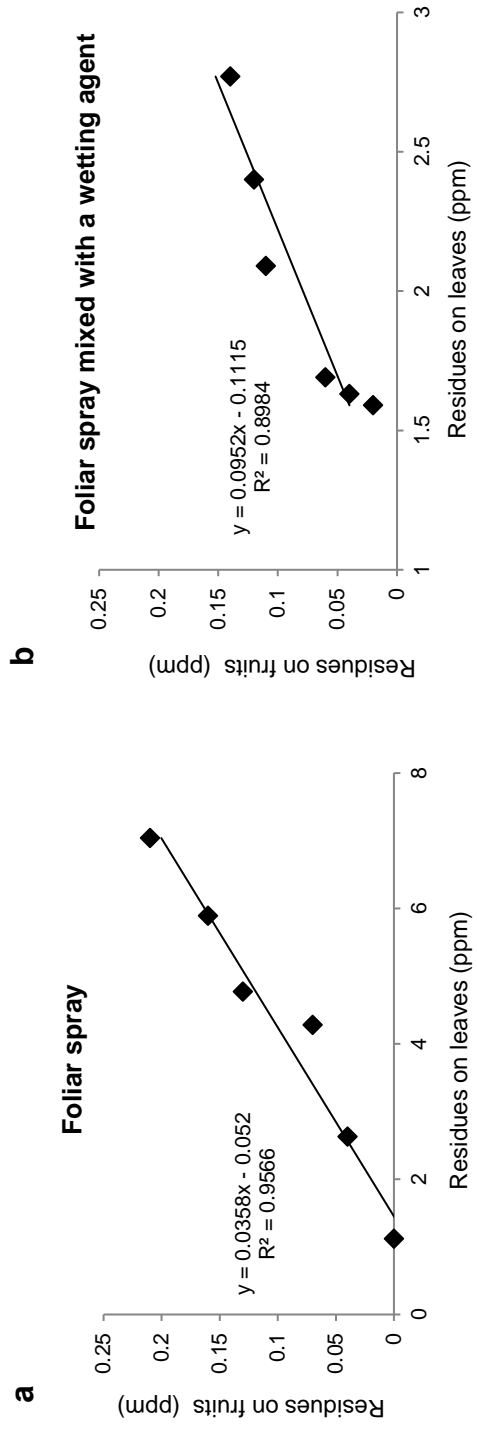


Fig. 4.4. Correlation of concentrations in leaves and fruits between foliar spray and foliar spray mixed with a wetting agent of chlorantraniliprole by UPLC/MSD.

4.4. Discussion

Conventional application methods of insecticides against arthropod pests include foliar applications at regular intervals. Evaluation of toxicities and residues of insecticides treated with foliar and soil drench applications provides useful information for controlling the pest population and to select effective application methods of the insecticides (Byrne et al., 2010).

In this study, toxicities of chlorantraniliprole were evaluated on red pepper treated with three application methods and UPLC/MSD was used to quantify the residue of chlorantraniliprole on leaves and fruits. The toxicity was more stable and the residue was higher in the foliar spray compared with the foliar spray mixed with a wetting agent at 28 days. The reason might be that the spray droplet mixed with a wetting agent could give low surface tension and spreading on plant leaves broadly increasing the adhesive and penetrating power on the plant surface and correlated to biological activity strongly (Taylor, 2011). The residue of chlorantraniliprole in the foliar spray showed lower compared with the foliar spray mixed with a wetting agent at 42 days. The reason might be that penetrating power in foliar spray is lower than foliar spray with a wetting agent because chlorantraniliprole with a low solubility in water (0.9-1.0 mg/L at 20 °C and pH 7) may not be penetrated alone without agent. The increase of toxicity and residue of

chlorantraniliprole in soil drenching may be due to systemic properties of root-uptake of chlorantraniliprole, and this is an option of application methods for the control of lepidopteran pests (Cameron et al., 2015). The systemic properties of the insecticide in the soil drenching shows the potential application method as a management tool. In monitoring of residues over time can provide valuable information for the use of soil drenching as an effective application method.

The residual concentration of chlorantraniliprole on fruits was significantly lower than on leaves due to the different composition and wettability of epicuticular wax layers between the different surfaces of fruits and leaves (Taylor, 2011; Holloway and Jeffree, 2017). In this study, we suggested that there are different characteristics among application methods, the foliar spray shows high toxicity, and the foliar spray mixed with a wetting agent and soil drenching show residual toxicities.

Chapter 5.

Insect pest management program with selective insecticides in greenhouses

5.1. Effective insecticides against *B. tabaci*, *S. litura*, and *F. occidentalis*

To select effective insecticides against major pests, *B. tabaci*, *S. litura*, and *F. occidentalis*, I conducted contact toxicity assay to all the life stages of *B. tabaci*, residual toxicity assay to *S. litura* larval stage, and contact and ingestion toxicity assay to *F. occidentalis* adult stage (chapter 2). Of various biotype of *B. tabaci*, Q biotype *B. tabaci* is the most widespread and dominant species in protected conditions (Kontsedalov et al., 2012). *Bemisia tabaci* was found to be resistant to neonicotinoid insecticides (Lee et al., 2012). However, 1 neonicotinoid (dinotefuran), 1 METI acaricide (pridaben), 3 avermectins (abamectin, emamectin benzoate, and lepimectin), and 1 spinosyn (spinetoram) were selected as the most effective insecticides to control Q biotype *B. tabaci* (Table 5.1). The cross-resistance of *S. litura* has been reported in worldwide (Ahmad et al., 2007; Muthusamy et al., 2014; Saleem et al., 2016). To reduce the development of resistance, ten insecticides (chlorantraniliprole, cyantraniliprole, flubendiamide, spinetoram, bistrifluron, bifenthrin, chlorfenapyr, chlorpyrifos, indoxacarb, and methoxyfenozide) in 8 different chemical groups can be treated by rotation (chapter 2 and Table 5.1). *Frankliniella occidentalis* is one of the most difficult pests to be controlled by insecticides due to the characteristics such as different ecological niche of each stage (Chung,

2001) and microhabitat preference in host plants (Lee et al., 2003). In this study, four insecticides (chlorfenapyr, emamectin benzoate, spinetoram, and spinosad) in three chemical groups were selected in contact and ingestion toxicity assay (Table 5.1).

Table 5.1. The effective insecticides selected against *B. tabaci*, *S. litura*, and *F. occidentalis*.

Target	Chemical groups (Mode of Action)					
	Avermectin	Diamide	MTI acaricide	Neonicotinoid	Spinosyn	Others
Q biotype <i>Bemisia tabaci</i>	Abamectin Emamectin benzoate Lepimectin	-	Pyridaben	Dinotefuran	Spinetoram	-
<i>Spodoptera litura</i>	-	Chlorantraniliprole Cyantraniliprole Flubendiamide	-	-	Spinetoram	Bistrifluron Bifenthrin Chlorfenapyr Chlorpyrifos Indoxacarb Methoxyfenozide
<i>Frankliniella occidentalis</i>	Emamectin benzoate	-	-	-	Spinetoram Spinosad	Chlorfenapyr

5.2. Selective insecticides against biological control species

For IPM practice based on biological control species, the contact and residual toxicities of 13 effective insecticides determined in chapter 2 were evaluated against 7 biological control species (predator mites, hymenopteran parasitoids, and hemipteran predators) in chapter 3.

After release of biological control species, six insecticides (bistrifluron, chlorantraniliprole, cyantraniliprole, dinotefuran, indoxacarb, and methoxyfenozide) and three insecticides (bistrifluron, chlorantraniliprole, and methoxyfenozide) were safe to predatory mites and hemipteran predators, respectively. One insecticide, chlorantraniliprole, was harmless to *D. isaea*. (Table 5.2).

Most of insecticides except chlorfenapyr were safe at 7 days before the release of predatory mites. However, for hymenopteran parasitoids, five insecticides (bistrifluron, chlorantraniliprole, cyantraniliprole, flubendiamide, and methoxyfenozide) were harmless. For hemipteran predators, nine insecticides (bistrifluron, chlorantraniliprole, chlorfenapyr, cyantraniliprole, emamectin benzoate, flubendiamide, indoxacarb, lepimectin, and methoxyfenozide) were safe.

Table 5.2. The selective insecticides against biological control species (predator mites, hymenopteran parasitoids, and hemipteran predators).

Target	Predator mites		Hymenopteran parasitoids		Hemipteran predators	
	After release	7 days before release	After release	7 days before release	After release	7 days before release
Q biotype <i>Bemisia tabaci</i>	Dinotefuran	Dinotefuran Emamectin benzoate Lepimectin Pyridaben	-	-	-	Emamectin benzoate Lepimectin
<i>Spodoptera litura</i>	Bistrifluron Chlorantraniliprole Cyantraniliprole Indoxacarb Methoxyfenozide	Bistrifluron Chlorantraniliprole Cyantraniliprole Emamectin benzoate Flubendiamide Indoxacarb Methoxyfenozide Spinetoram	Chlorantraniliprole	Bistrifluron Chlorantraniliprole Cyantraniliprole Flubendiamide Methoxyfenozide	Bistrifluron Chlorantraniliprole Methoxyfenozide	Bistrifluron Chlorantraniliprole Cyantraniliprole Emamectin benzoate Flubendiamide Indoxacarb Methoxyfenozide
<i>Frankliniella occidentalis</i>	-	Emamectin benzoate Spinetoram Spinosad	-	-	-	Chlorfenapyr Emamectin benzoate

5.3. Management programs to control of *B. tabaci*, *S. litura*, and *F. occidentalis*

Pest management strategies can be complex to provide a good balance between the biological control and chemical control. The augmentative biological control can be adopted to manage pest density below damage level or during early pest occurrence. When the pest density exceeds the damaging level, the chemical control may be adopted to reduce the pest density rapidly. Due to the direct damage by sucking of nutrients and indirect damage by transmitting more than 100 viral diseases, the populations of *B. tabaci* should be controlled during the early occurrence (Byrne, 1999; Lee et al., 2005). The chemical control should be adopted cautiously to control *B. tabaci* in biological control scheme because of the direct contact toxicity of the insecticides to natural enemies.

Spodoptera litura has occurred on various fruit and leaf vegetables and caused direct yield loss in greenhouse cultivation system (Lim et al., 2012). Fruit damage of sweet pepper was highly correlated with 2nd instar larvae of *S. litura* by causing 1, 3, 5% of damaged-fruit in 0.2, 0.5, 0.8 larvae per plant, respectively (Park et al. 2010). The insecticide resistance of *S. litura* has been reported in worldwide (Ahmad et al., 2007 and 2008;

Muthusamy et al., 2014; Saleem et al., 2016). To reduce the development of resistance, the chemical and biological controls should be compatible in IPM systems and insecticides with different mode of action should be rotated.

Frankliniella occidentalis has complex life cycle, i.e., different ecological niche of each life stage, and can cause direct damage in the developing fruit, such as eggplant and pepper, by feeding on and laying eggs (Chung, 2001; Lee et al., 2003). The economically tolerable rate of fruit damage based on control cost and market values under greenhouse cultivation was estimated as < 8.0%. Economic thresholds of *F. occidentalis* were < 2.1 adult or nymphal stages per flower and < 5.7 adult stages per four-day sticky card count in unripe red pepper (Park et al., 2007). Farmers are relying on chemical controls, but experiencing failure of control due to the different ecological niches of the life stages of thrips and the development of insecticide resistance. However, the population of *F. occidentalis* could be managed with two complementary strategies of selective insecticides and biological control species to reduce sustainably the population in crop with controlling resistance and preventing invasion of thrips from weeds and wild vegetation (Allsopp, 2010).

To reduce the yield loss and the development of resistance in greenhouse cultivation system, management programs of *B. tabaci*, *S. litura*, and *F. occidentalis* were established with the use of selective insecticides found in Chapter 2 and 3 and application methods in Chapter 4 illustrated as in Fig. 5.1, 5.2, and 5.3, respectively.

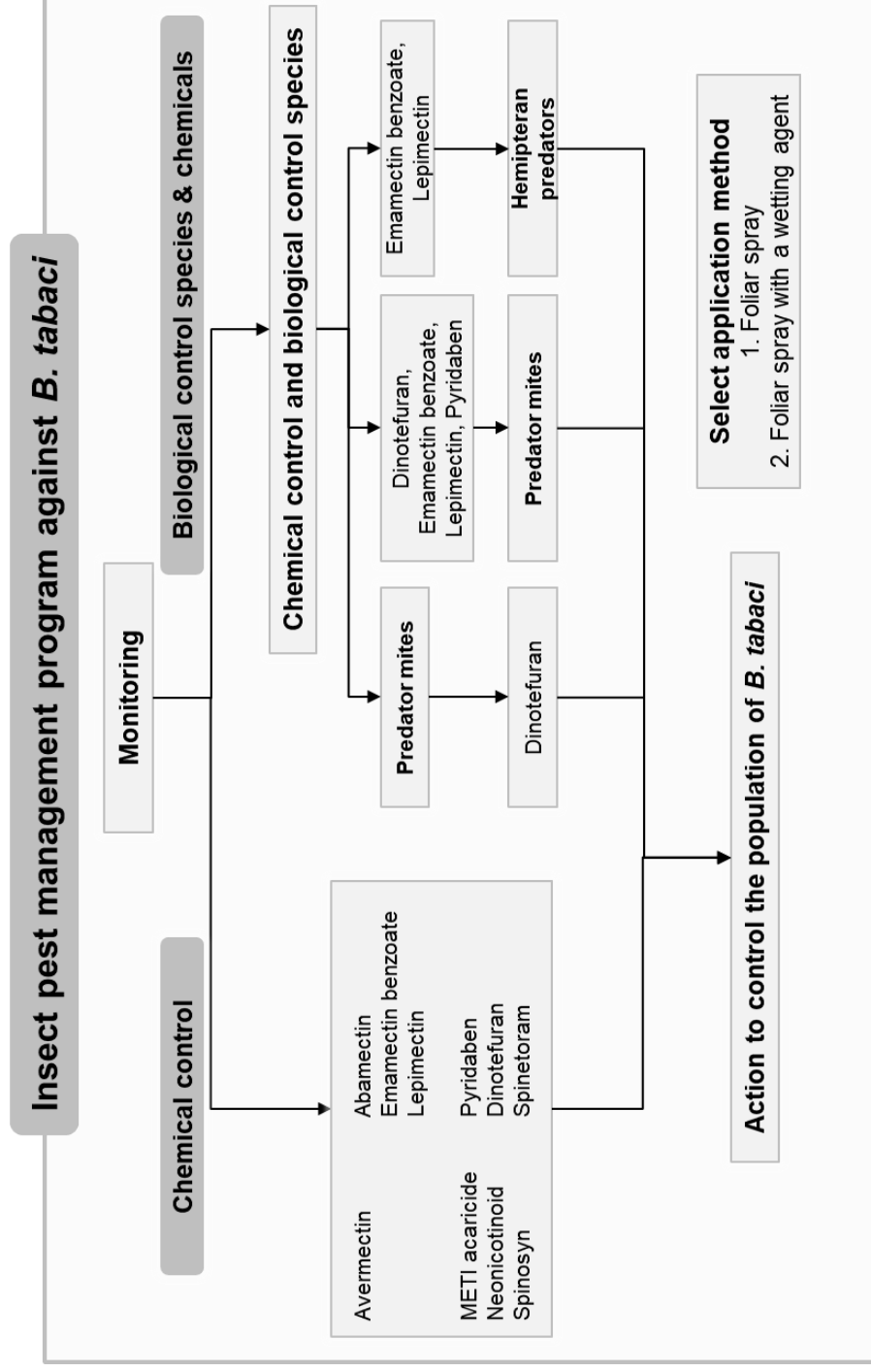


Fig. 5.1. Flow chart of management program for controlling *B. tabaci*.

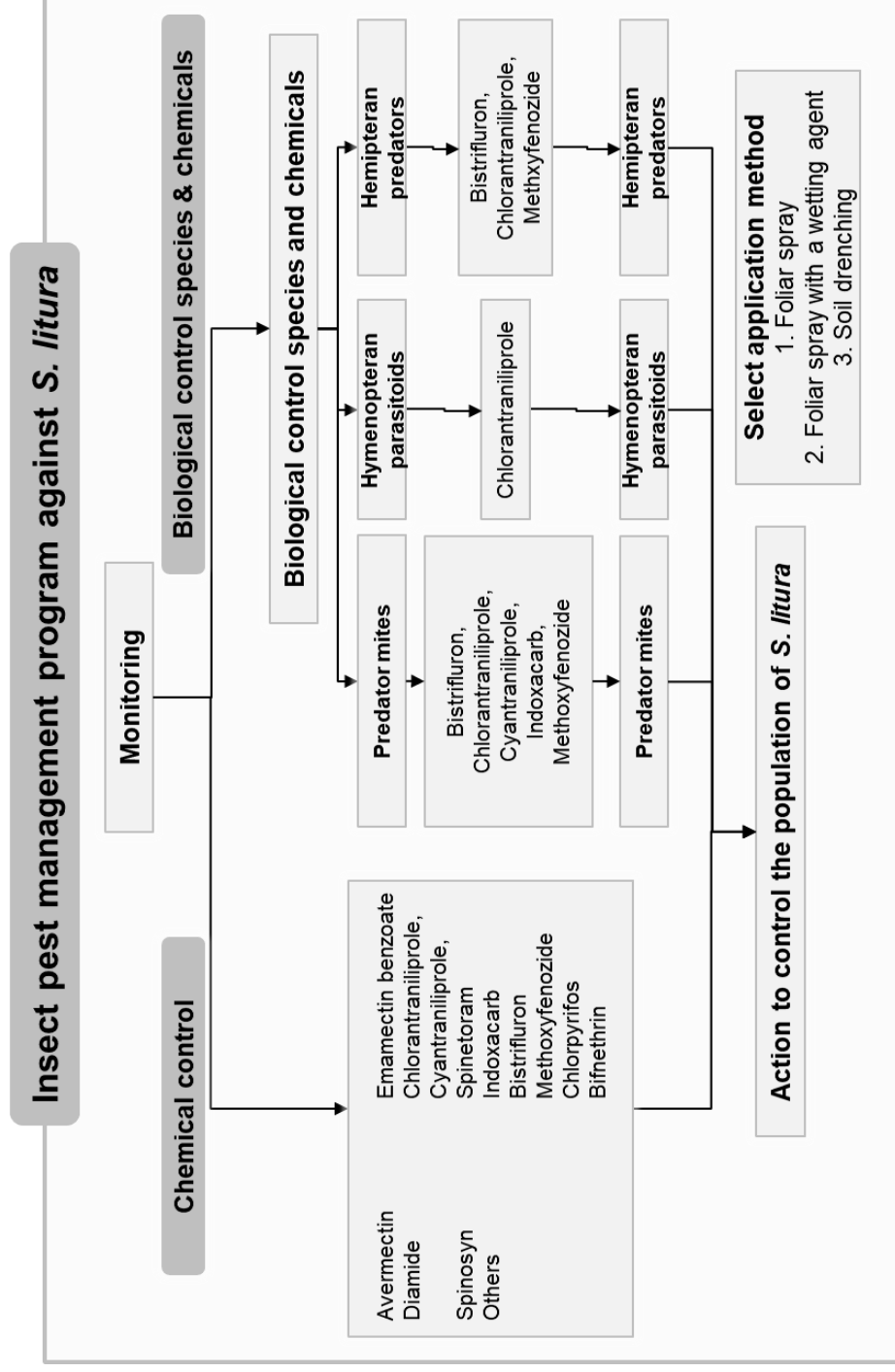


Fig. 5.2. Flow chart of management program for controlling *S. litura*.

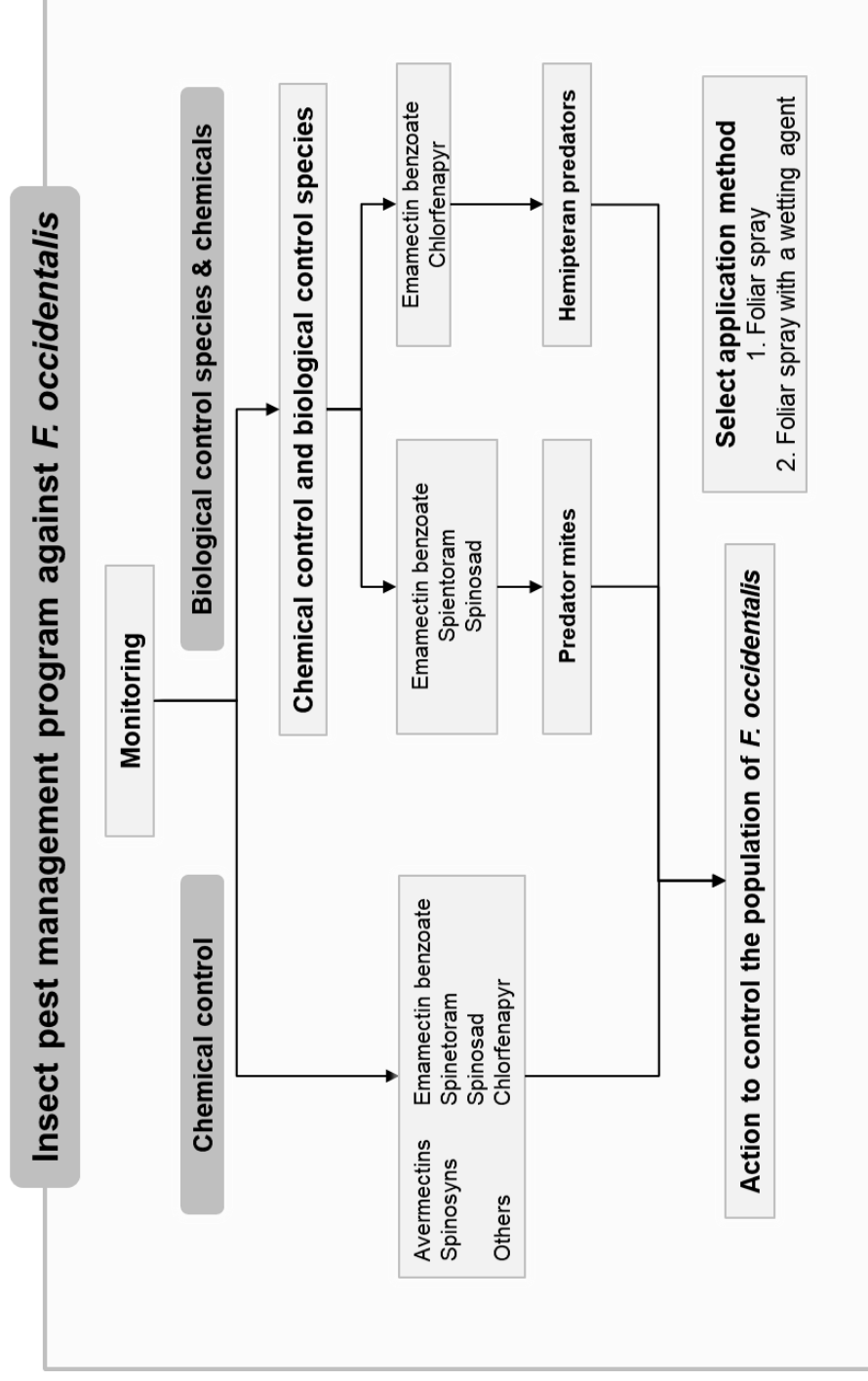


Fig. 5.3. Flow chart of management program for controlling *F. occidentalis*.

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국문 초록

시설재배에서 선택적 살충제를 이용한 담배가루이, 담배거세미나방 및 꽃노랑총채벌레의 화학적 방제 프로그램

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시설작물의 주요해충인 담배가루이, 담배거세미나방, 그리고 꽃노랑총채벌레의 방제는 작물 생산량 증대에 핵심적인 요소이다. 이들 해충의 밀도와 저항성 관리를 위하여 화학적 방제와 생물학적 방제의 상호보완적 방제전략이 요구되어 왔다. 따라서 본 연구에서는 살충활성이 우수하며 천적들에 안전한 선택적 살충제를 선발하고, 살충활성을 높일 수 있는 효과적인 처리방법을 선발하여 종합해충관리(Integrated Pest Management) 체계를 제안하였다.

살충제의 작용기작별로 우수한 약제를 선발하기 위하여 담배가루이의 알 및 약충과 성충, 담배거세미나방의 유충, 꽃노랑총채벌레의 성충에 대하여 살충활성을 평가하였다. 담배가루이의 전생육단계에 대하여 3 종의 avermectins (abamectin, emamectin benzoate, lepimectin), 1 종의 METI (pridaben), 1 종의 neonicotinoid

(dinotefuran), 1 종의 spinosyn (spinetoram)이 우수한 살충제로 선발되었다. 담배거세미나방에 대한 잔효독성을 평가한 결과 4 종(chlorantraniliprole, cyantraniliprole, flubendiamide, indoxacarb)은 21 일간 지속되어 우수한 살충제로 선발되었고, 6 종(bistrifluron, chlorfenapyr, methoxyfenozide, chlorpyrifos, bifenthrin, spinetoram)은 7 일까지 살충활성이 지속되어 저항성 관리를 위한 교호살포제로 선발되었다. 꽃노랑총채벌레에 대한 접촉과 섭식독성을 평가한 결과 3 종(spinetoram, spinosad, emamectin benzoate)은 접촉독성이 우수하였고, chlorfenapyr 은 섭식독성이 우수한 살충제로 선발되었다.

천적과 상호보완적으로 활용할 수 있는 살충제를 선발하기 위하여 13종의 살충제를 천적 7종(포식성 응애류 2종, 포식성 노린재류 2종, 기생성 쯤벌류 3종)에 대한 접촉 및 잔효독성을 평가한 결과 포식성 응애류에 대해 6 종(dinotefuran, indoxacarb, chlorantraniliprole, cyantraniliprole, methoxyfenozide, bistrifluron)이, 포식성 노린재류에 대해 3 종(methoxyfenozide, bistrifluron, chlorantraniliprole)이 안전한 살충제로 선발되었다. 그러나, 기생성 쯤벌류에 대해서는 대부분의 약제가 독성이 있었으나, 굴파리쯤벌에는 chlorantraniliprole 이 안전한 살충제로 선발되었다.

효과적인 처리방법을 선발하기 위하여 고추에서 chlorantraniliprole 의 살충활성 차이와 작물 잔류량 분석을 통하여 3 가지 처리방법(경엽처리, 전착제 혼용처리, 관주처리)을 평가하였다. 살충활성은 경엽처리 및 전착제 혼용처리, 관주처리 순으로 나타났으나, 경엽처리제는 다른 처리방법에 비하여 잔류량이 빠르게 감소하였다. 살충활성과 작물잔류량은 경엽처리와 전착제 혼용처리에서 42 일까지

안정적으로 유지되었다. 관주처리 방법의 경우 처리 후 3 일까지 낮은 작물 잔류량과 살충활성이 나타났으나, 7 일부터 42 일까지 높은 잔류량과 살충활성을 유지하였다. 경엽처리는 약제처리 직후의 높은 살충활성으로 해충이 다발생하는 시기에 효과적이며, 관주처리는 처리 3 일 이후에 살충활성이 관찰되므로 해충의 발생초기에 활용하는 것이 효과적일 것이다.

본 연구에서 담배가루이에 대해 6 가지, 담배거세미나방에 대해 10 가지, 그리고 총채벌레에 대해 4 가지의 살충제가 선발되었으며, 천적 7 종(포식성 응애류 2 종, 포식성 노린재류 2 종, 기생성 쯤벌류 3 종)에 대한 접촉독성과 잔효독성에서 chlorantraniliprole 이 가장 안전한 약제로 선발되었다. 살충활성과 잔효력이 우수한 chlorantraniliprole 을 활용하여 해충을 방제하는 경우 가장 효과적인 처리방법은 전작제를 혼용하지 않고 chlorantraniliprole 을 경엽처리하는 것이었다.

주요어: 종합해충방제, 선택적 살충제, 살충활성, 저항성, 천적, 처리방법

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